Nonclinical Assessment of Potential Hepatotoxicity in Man

November 2000

Introduction

Severe hepatotoxicity is one of the most common causes for pharmaceutical product recalls, labeling changes and Dear Doctor Letters which raises the question of how effective nonclinical and clinical testing are in recognizing such toxicity. A primary purpose of nonclinical studies is to discover target organ toxicity and from this information stop the development of the compound or to utilize this information for monitoring possible toxicities in human studies. The liver is a major target organ of early screening efforts in the pharmaceutical industry and a major target organ in the repeated dose nonclinical safety studies used to support clinical trials. If a compound is hepatotoxic in animals, it is only after the toxicity is assessed and an adequate safety margin is estimated that such a compound is administered to humans. Despite these precautions, hepatotoxicity may be identified in clinical trials or sometimes during marketing. In some of these instances hepatotoxicity may have been observed in nonclinical studies but not judged significant, or in others, signals may have been inapparent.

This document was prepared by an FDA Working Group in consultation with representatives of PhRMA and AASLD. It is not a guidance document. It does not contain recommendations to sponsors or applicants regarding particular actions they should take. In stead it is a concept paper that assesses the current state of knowledge, and the existing methodology for examining hepatotoxic events associated with pharmaceuticals. It is meant to provide a framework for discussion at a public workshop on drug-induced hepatotoxicity to be held February 12-13, 2001. As the knowledge evolves about this topic, the Agency may decide to develop guidance on how sponsors can better identify drugs that cause hepatotoxicity during the pre-clinical, clinical, and post-marketing periods.

It is presumed that most problems that have arisen in humans are due to low frequency idiosyncratic effects, metabolic variability in man, or unidentified interactions with other agents or factors. Unfortunately, none of these presumptions are well documented. Although much attention has been focused on the predictivity of animal models for clinical findings in humans, in fact clinical trials of pharmaceuticals are a relatively poor source of information for ascertainment of "predictivity" (probability that an event observed in an animal model is also observed in the human) because clinical trials are designed specifically to avoid adverse outcomes that have been observed in animal models. "Negative predictivity" (probability that an event not observed in an animal models will also not be observed in the human) is more readily determined, but even in this case such data on hepatotoxicity do not seem to be readily available. Of critical importance to avoiding unanticipated hepatotoxicity in man is the need to understand why drugs that were judged safe to administer to man on the basis of animal data sometimes cause unexpected hepatotoxicity. In other words, why do animal models, in these cases, fail to predict human hepatotoxicity? It is probably even more important to understand why these models sometimes fail to identify hepatotoxic potential in humans than to fully define the overall effectiveness of animal studies in predicting human outcome. This question can be addressed using the techniques of "failure analysis". If the reasons for failure of nonclinical models to predict human hepatotoxicity could be determined, then better testing approaches should become evident.

Presently, there is a lack of sufficiently organized data to make an informed conclusion on the predictivity of nonclinical studies for identifying the risk of significant hepatotoxicity in clinical trials and in the postmarketing population. To address this issue it is necessary to determine whether there are data that would allow accurate predictions to be made, whether there are early signals that indicate a drug will cause serious hepatotoxicity in humans, and whether there are signals that indicate that the hepatotoxicity of the drug poses an acceptable risk.

For compounds that have progressed into human trials and have then caused severe human toxicities, it needs to be determined whether patterns existed in the nonclinical studies that signaled these events. In cases where no signals can be identified in the animal studies, the reasons for this nonpredictivity need to be determined. Possible reasons include 1) differences in metabolism between the animal model and man that lead to the generation of an active metabolite in man at levels much greater than in the nonclinical model, 2) immune-mediated idiosyncratic responses, 3) predisposing factors in man that didn't exist in the animal model, such as ethanol-induced metabolic sensitivity to acetaminophen hepatotoxicity, or 4) other factors. To answer many of these questions, data must be gathered from assessments of hepatotoxicity by the pharmaceutical industry.

Immune-mediated idiosyncratic drug reaction has been responsible for numerous serious hepatotoxic events in humans and poses special problems from the perspective of nonclinical hepatotoxicity

evaluation. It represents an example of a genetically based reaction occurring in selective individuals and may not be readily identified in current nonclinical studies. For such responses, development of nonclinical testing models is an unmet challenge. This is a hypersensitivity reaction which is accompanied clinically with rash, fever, eosinophilia, and multiple organ system involvement. The initial duration of exposure required to produce these reactions varies from 1 to 5 weeks, while the response to rechallenge with the drug is immediate and can be serious. Phol and associates estimated that immune-mediated type of idiosyncratic reactions may account for between 3-25% of all idiosyncratic drug reactions.[1] The sequence of events leading to immune-mediated drug injury are hypothesized as follows:

Most drugs that are associated with immune-mediated reactions are not known to be sufficiently reactive in themselves to act as drug haptens and form drug-carrier conjugates, which can then interact with the host's immune system. Consequently, it is proposed that drugs which act as immunogens, are first biotransformed by the liver, converted to haptenic metabolites, which then form drug-carrier conjugates. Once the conjugate is formed, it may act as a neoantigen or immunogen and elicit a specific antibody-mediated or cell-mediated immune response. In some cases, both types of responses are elicited.

> Immune-mediated mechanisms have been proposed for idiosyncratic reactions observed with several drugs including: halothane,[2, 3] phenytoins,[4] sulfonamides,[5] and tienilic acid[6] to name a few.

This document will assess 1) the current nonclinical practices used for assessing potential hepatotoxicity in man, 2) predictivity of nonclinical studies for human hepatotoxicity, and 3) the consequences of hepatotoxicity on drug development.

Methodology

Nonclinical assessment of drugs for hepatotoxicity is done in a tier approach. Tier I are standard screening studies and tests designed to detect and initially characterize a hepatic change, while tier II are specialized studies and tests used to further characterize a change or address a specific nonclinical or clinical safety issue.

Tier I toxicity studies are conducted in standard animal models using both dose and duration multiples above those anticipated for clinical trials and treatment of the proposed patient population. In each nonclinical animal study, multiple doses are selected to produce both dose-limiting toxicity and a *no effect level* (NOEL). Equally important, is the determination of a *no adverse effect level* (NOAEL). Risk assessment of a given toxic effect in humans is usually based on the safety margin of the compound, i.e. the ratio of the NOAEL in the most sensitive species and the anticipated therapeutic dose[7] . In tier I nonclinical studies, a broad range of parameters are evaluated at various time points to insure detection and characterization of hepatic changes. Tier II studies and tests will not be discussed in detail, since they may vary considerably depending on the issue to be addressed. It is in this area that most of the efforts for the identification of immune mediated responses have been directed.

Tier I studies

Nonclinical assessments typically include administration of drug to two laboratory animal species (1 rodent and 1 nonrodent - usually dog/monkey) for multiple durations and doses exceeding those used in clinical trials. The duration of nonclinical chronic toxicity studies generally has been 6 months in rodent and 1 year in dog/monkey. Reversibility of toxicity is evaluated by including animals in high dose and control groups which are left untreated for 2 to 4 additional weeks. This is applied to studies of 1 to 3 months duration. However, the duration of the reversibility period and the nonclinical studies to which it is applied may vary, depending on many factors, including the time it takes to induce the liver change and the anticipated time for which it takes to demonstrate reversibility. Some drugs necessitate additional nonclinical testing in special animal models when these animal models more appropriately mimic the sensitivity of man to certain classes of hepatotoxins, e.g., woodchuck for nucleoside analogs. The duration of nonclinical studies is dependent on multiple factors, including: 1) duration and dosing regimen of the clinical trial; 2) the drug's chemical structure and its relation to known toxic entities or liabilities; and, 3) regulatory guidance. The use of one, two or more animal species is dependent on: 1) the relevance of the animal model for predicting human toxic potential; and, 2) regulatory guidance. Tier I nonclinical studies are conducted in normal animals, assuming extrapolations of hepatotoxic potential can then be made to both normal and health-compromised humans.

Tier I parameters

Tier I parameters used for detection of hepatic changes are clinical pathology, morphologic pathology, enzyme induction, and in-life findings. All but enzyme induction should be assessed in every study. These parameters are determined at phases of the study that allow identification of acute, chronic, persistent, transient and/or reversible hepatic change. Clinical and morphologic pathology evaluations are the 'gold standard' for identification of hepatic toxicity in animals, each complimentary to the other and each with its advantages (Appendix: Table 1). Additional tier II assessments may be made with ultrastructural pathology, morphometrics, histologic special stains, or methods for antibody detection.

Early Screening Studies

In most pharmaceutical companies, specific screening for hepatoxicity is usually used when liver toxicity has been identified as a major limiting toxicity for the class compound[8, 9]. If a large number of analogues are available such screening may be implemented to select a compound with an optimized profile. Three approaches will be discussed: (a) *in vitro* cytotoxicity screening assays, (b) *in vitro* covalent binding assays, and (c) toxicogenomics/proteomics assays.

In Vitro Cellular Assays

Cultured hepatocytes are the most commonly used *in vitro* cytotoxicity test system[10] . Precision cut liver slices are also implemented for this screening[11, 12] . A major advantage over cultured hepatocytes is that precision cut liver slices can be easily prepared from various species, including humans, with the same technique[13]. Ideally, the *in vitro* test should be mechanistically based so that the toxicity parameters assessed in vitro are related to the mechanism of toxicity *in vivo*[9] (e.g., β oxidation if the compound induces mitochondial toxicity). More often, the mechanism of hepatoxicity is not known and the *in vitro* model is selected for the type of hepatotoxicity observed. For hepatocellular necrosis, a conventional model (e.g., cultured hepatocytes or liver slices) and crude cytotoxicity or cell death endpoints (i.e., enzyme leakage, dye exclusion) can be considered toxicologically relevant[13]. More complex models, such as hepatocyte couplets with specific end points (e.g., bile acid or fluorescin secretion) can be applied for drug-induced cholestasis) [14, 15, 16].

Covalent Binding

Several *in vitro* methods are available to detect and quantify binding for a drug or its metabolite(s) to liver proteins including radiochemical and immunological methods[17]. A large number of studies indicate that drug-induced liver injury correlates with covalent binding of the drug to specific proteins[18, 19]. Immunochemical techniques have been used to identify the target protein adducts of several hepatotoxicity compounds[20, 21, 22]. These studies have shown that reactive metabolites bind selectively to certain proteins. Acetaminophen metabolites have been shown to selectively bind a microsomal 42-44 k Da protein and a cytosolic 56-58 k Da protein suggesting that cell death may result from selective alkylation of critical intracellular proteins[21, 22]. Also, alkylation of certain proteins has been suggested to lead to formation of neoantigens that may trigger immunemediated hepatotoxicity[23]. Supporting this concept is the example cited above where it has been shown that patients with tienilic acid-induced hepatitis have anti LKM_2 antibodies in their serum which strongly recognizes P450 2C9, the main liver microsomal protein covalently bound by tienilic acid metabolites[23, 24].

If direct as well as immune mediated hepatotoxicity can be shown to be initiated with covalent binding to certain proteins, it may be possible to develop predictive tests for certain hepatotoxicities.

Genomics/Proteomics

Gene expression is altered in most cases of liver toxicity by either direct or indirect mechanisms. The challenge facing toxicologists is to define and identify the change in gene expression elicited by a toxin. Microarray technology, or other genomic or proteomic approaches, may allow detection of these changes in gene expression and may be a foundation for a fundamental

approach to toxicology testing[25]. Nucleotide array platforms are based on c DNAs or oligonucleotides immobilized on a solid support. For hepatotoxins, probes are then generated from RNA harvested from hepatocytes which were exposed to the test agent *in vitro* or *in vivo*. These are then hybridized on a microarray and the pattern of the probes is read as an index of gene expression. Discrepancies between microarrays from control and treated hepatocytes may indicate drug-induced changes in gene expression. By testing hepatotoxins of known mechanisms, a signature may be developed for that mechanism of toxicity. Compounds of unknown activity could thus be screened for potential hepatotoxicity by comparing signatures of gene expression. Proteomics is an approach where the product of altered gene expression, proteins, are harvested from exposed cells/tissues and characterized by such methods as 2 D gel electrophoresis, protein arrays, or laser desorption techniques. The signature of a toxicant could also be identified. Such a system could be useful for screening compounds for hepatotoxicity or for defining mechanisms of toxicity much like the microarray technology. Covalent binding of compounds or metabolites to proteins can also be identified by these methods. These surrogates for toxicity may become a powerful tool for predicting toxicity and for defining the mechanism of toxicity. This field is rapidly evolving and several companies are at an early stage of implementation. One remaining problem is how to deal with the toxicity related to various specific genetic polymorphism in human responses.

Data Interpretation

The purpose of data interpretation is to determine if hepatobiliary changes occurred, identify and characterize theses changes and their magnitude, determine a NOEL and NOAEL, and determine a mechanism/pathogenesis for the changes.

Hepatic changes are identified by comparison of data in animals given drug to the appropriate controls, and from understanding the utility of group mean and individual animal data. For rodents, the most appropriate controls are concurrent controls included in the study; historical and published control data are of benefit in that they identify a framework from which to operate. Given the homogeneity of rodents, group data generally are as or more important in making assessments than individual animal data. For dogs, and even more so with nonhuman primates, changes must be identified when compared to pretreatment data (clinical pathology only) combined with concurrent control and historical control data. Published control data have limited value. Dogs and nonhuman primates are more heterogeneous than rodents and the numbers of animals used on study is fewer than with rats. Thus individual animal data are as, or more important than group mean data.

Interpretation should be made by experts demonstrating high level understanding of the disciplines and the relationship of liver injury to other occurrences in the whole animal. Data need to be analyzed,

reported and interpreted *in toto*, with emphasis on correlating clinical and morphologic pathology data. Additionally, critical information includes identification of pathogenesis and/or mechanism of how the drug induces changes in the liver. This is especially useful as it provides perspective as to the implication of hepatic changes in man.

Determination of 'adverse' for calculation of NOAEL is multifactorial, and is based on a combination of 'parameters affected and their magnitude of change' and 'mechanism of change'. It is difficult to identify an absolute cut-off between adverse and not adverse for an individual parameter. However, certain findings in individual parameters are considered adverse, unless they can be associated with a mechanism that is non-adverse (Appendix: Table 2).

Changes in liver can be classified a number of different ways, but the two most useful are classification by the 1) Process or 2) Function affected (Appendix: Tables 3 and 4). Clinical and morphologic pathology findings are used to classify hepatic changes and must be correlated to make accurate assessments. Categorization by Process is primarily a histopathologic classification. As a result of the morphologic changes to the liver, there are functional changes, which allows classification primarily by clinical pathologic changes.

The hepatotoxicity must be assessed in context of onset, severity, duration and reversibility. The plasma drug (and metabolite) concentrations should be determined at the NOAEL as well as at toxic doses and these are compared to anticipated or achieved plasma concentrations in humans at the highest therapeutic doses.

Use of Nonclinical Data in Safety Assessment

Data and interpretations from nonclinical studies are communicated to clinicians. These data allow clinicians and regulatory agencies to determine the margin of safety, the impact of similar lesions in the patient population, whether the risk is acceptable for the therapeutic effect of the drug and for the patient population, and how to monitor for the onset of adverse effects. Depending on the definition of hepatotoxicity, not all hepatotoxicities are adverse. Not all changes in the liver are adverse. Thus, determination of which changes are adverse and which are not will impact the development of a drug. The clinical approach to drugs that cause obvious adverse toxicities, e.g., massive hepatocellular necrosis or acute liver failure, is different than the clinical approach to a drug that causes a non-adverse adaptive change, e.g., peroxisome proliferation in rats. Similarly, hepatic toxicities that occur by a mechanism that indicates premonitory signs will precede adverse changes are addressed differently than toxicities in which there are no premonitory signs.

Not all hepatoxicities can be adequately monitored in clinical trials. Those that can not be monitored must have a pathogenesis indicating it is not adverse in humans and/or occur at a dose providing a very large margin of safety. For hepatotoxicities that can be monitored, e.g., hepatocellular degeneration/necrosis resulting in leakage of hepatocellular enzymes, the induced lesion must be detectable and reversible before progression to a severe state and must occur at a dose that provides an adequate margin of safety.

Predictive Value of Nonclinical Studies

The data on predictivity of animal studies for human toxicities have been limited and a complete analysis has not been conducted. However, experiences within the pharmaceutical industry indicate that the appropriate identification of hepatotoxicity is high. Most compounds found not to be hepatotoxic in animals are not hepatotoxic in humans. However, most compounds that are severely hepatotoxic in animals never proceed to clinical trials as it is judged that there is unacceptable risk for hepatotoxicity in man. The lack of information on the human correlate for these compounds prevents the predictivity of the animal models to be calculated. Although the animal models are thought to be highly effective in keeping most hepatotoxic compounds from being tested in humans, failures do occur where hepatotoxicities are observed in the clinic despite thorough testing by these models. The underlying reasons for the misidentification of an adverse effect to be of a low risk to humans need investigation. A limited number of surveys studying these failures have been done. In one survey where liver toxicity was the cause of project termination for 7 compounds after testing in humans, hepatotoxicity in animals was demonstrated in 4 of these compounds[26]. For the remaining 3 compounds, liver toxicity was not observed in the animal studies. Table 5 presents the results of a more recent and much larger survey[27] in which, 31drug-induced hepatotoxicities were reported in humans. Of the 31 hepatotoxicities, 18 were reported to have been detected in animal studies. Of these 18 hepatotoxicities, 10 were observed in rodents and 16 were observed in nonrodent (dog and/or primate) studies. Eight of the positive correlates would have been missed in rodent only assessments; however, 2 of the correlates were reported to have been observed in only rodent studies. Development of 1 of these 2 compounds was later terminated due to hepatoxicities observed in humans. Only 8 of the 18 positive correlations were observed in both rodent and dog studies. Of the 18 compounds hepatotoxic in animals, at least 15 were considered positive within one month of exposure. None were listed as positive after a single dose but it was possible that hepatotoxicity occurred but was not detected as there was no indication of parameters measured for the detection of hepatotoxicity. In this same survey, of 15 compounds terminated for human hepatotoxicity, 7 of these toxicities were predicted by animal models. For these 15 compounds, this toxicity was first observed in phase I trials for 7 compounds and phase II trials for 8 compounds. Fifteen of the compounds were specifically defined as not terminated by the observed human hepatotoxicity and animals predicted 11 of these toxicities. For these 15 compounds, 6 of these toxicities were first observed in phase I, 4 in Phase II and 5 in Phase III. The phase of clinical development in which human hepatotoxicities were first

observed is presented in Table 6. However, duration of treatment was not indicated and those for which hepatotoxicity did not occur until Phase II or Phase III studies, factors other than duration, such as larger numbers of humans treated could have influenced these results.

These data were also analyzed to determine if hepatotoxicity in humans was more likely to terminate compound development if there was a positive animal correlate for this toxicity. Of 18 compounds found hepatotoxic in clinical trials for which there was a positive animal correlate for hepatotoxicity, 16 were terminated due to this hepatotoxicity in humans. On the other hand, hepatotoxicity in humans resulted in termination of development for 8 of 13 compounds hepatotoxic in clinical trials but found to have no animal correlate for hepatotoxicity.

Of 28 compounds being developed by Rhône-Poulenc Rorer between 1988 and 1994, 10 induced liver changes in animals. Of the 10, 7 were tested in humans. Only 1/7 was found to cause liver injury in clinical trials. The remaining 18 compounds were negative for hepatotoxicity in animal studies. Fifteen of these were tested in man. In 5/15 of these compounds, liver injury was observed in clinical trials and in 2, liver toxicity led to project termination[13]. In the Rhone-Poulenc Rorer survey, none of the hepatotoxicities in humans produced serious liver damage. This information is not available for the other 2 surveys.

Eli Lilly reviewed 13 of its drugs in various stages of development that had hepatic findings in nonclinical studies. Five drugs were terminated due to nonclinical hepatic findings for which there was a lack of an adequate margin of safety for clinical trials; 3 of these 5 drugs also had hepatic changes which could not adequately be monitored in clinical trials. One drug was terminated following phase I clinical trials in which hepatotoxicity occurred in humans; nonclinical studies did not adequately identify the occurrence of this hepatotoxicity due to marked sensitivity of humans compared to standard laboratory animal models. Two drugs were developed to market, since there was a suitable margin of safety and the hepatic change in animals was not adverse. Two drugs had development terminated for reasons other than hepatotoxicity. Three of these 13 drugs are currently in development; 2 of these 3 have an adequate margin of safety for hepatic effects, while one has a narrow margin of safety but the life-threatening clinical indication combined with adequate clinical monitoring tests for hepatotoxicity allowed development.

As demonstrated by the surveys, there are compounds which show no significant evidence of liver toxicity in animals but cause liver toxicity in humans. These false-negative results in animal studies may be related to insufficient systemic exposure to the drug either because the doses were too low or because intestinal absorption was poor or the metabolism of the compound or the route of elimination differed in the animal species tested compared to humans[28] . The occurrence of other toxicities or extensions of the pharmacological effect may have prevented testing the compound at a hepatotoxic dose in the animals.

The small number of animals used in toxicity studies (10-20 rodents/sex/group; 3-6 non-rodent/sex/group) may make it difficult to detect hepatotoxicity occurring at a low incidence. The normal animals in nonclinical studies do not mimic the patient population or the potential drug:drug interactions which may occur in humans which further complicates interpretation of nonclinical study results. Finally, immunoallergic or auto-immune type liver toxicity can only rarely be accurately evaluated in nonclinical studies.

The Rhône-Poulenc Rorer data[13] also demonstrate that compounds eliciting hepatotoxicity in animals do not always cause hepatotoxicity in all animal species tested or in humans. Besides the species differences, there are other factors which may result in these false positives such as higher doses used in nonclinical studies compared to clinical trials.

As an example of a compound demonstrating species differences in severities of hepatotoxicity, lovastatin during nonclinical safety evaluation was well tolerated in dogs, rats and mice at doses which produced lethal centrilobular hepatic necrosis in rabbits[29]. The specific activity of HMG-COA reductase in rabbit liver is 10-200 times lower than in the rat, mouse and dog making it more susceptible to this toxicity. Thus toxicity in rabbits was completely blocked by coadministration of mevolonate, the product of the inhibited HMG-COA reductase. Although lovastatin is associated with low incidences of transient transaminase elevations in humans, it is not associated with serious hepatotoxicity in humans. In this example, elevations in transaminases observed in human trials were accurately predicted by an animal model, but the serious hepatotoxicity observed in the rabbit was not observed in humans. This lack of correlation in severities of hepatic effects occurring in rabbits and in humans could be a result of differences in species specificity in sensitivity to hepatotoxicity induced by lovastatin, differences in doses administered or a combination of the two. The dose producing severe hepatotoxicity in rabbits was 200 to 1000 times the therapeutic doses recommended for humans.

No surveys have been done to determine the predictivity of nonclinical studies for a lack of human hepatotoxicity. This is valuable information as it is included in the assessment used in designing the clinical testing program.

Consequences of Hepatotoxicity on Drug Development

Liver toxicity in animals or in humans does not necessarily lead to cessation of drug development. Of 28 NCEs under development by Rhône-Poulenc Rorer between 1988 and 1994, 10 caused liver changes in animal toxicity studies (liver enlargement 7/10, hepatocellular necrosis 3/10, cholestasis 1/10). Of these 10, development of one was stopped because of marked enzyme induction potential while 2 other compounds were terminated for reasons other than toxicity. Clinical studies were conducted with the remaining 7 compounds[13]. As liver toxicity is usually reversible and it can be monitored non-invasively

in the clinic by sensitive clinical pathology parameters, the risk to proceed to human clinical trials with a compound shown to be hepatotoxic in animals is considered manageable if an appropriate therapeutic index is possible at study initiation. However, hepatotoxicity in clinical studies is a major cause of compound termination for safety reasons. In a survey on 320 NCEs from 18 companies, 29 were terminated because of adverse effects in humans[26]. Effects on the liver were the principal cause of termination for 9 of these 29.

In a recent survey[27] where 84 development compounds were listed as being terminated due to clinical toxicity, 15 were terminated due to human hepatotoxicity. This was second only to cardiovascular toxicity which was responsible for the termination of 18 compounds. In this same survey 15 compounds found hepatotoxic in humans were not terminated for toxicity. How many of these compounds proceeded to marketing was not part of this survey. Neither were data which would distinguish which markers of hepatotoxicity in nonclinical trials correlated with termination of development due to human hepatotoxicity.

Hepatotoxicity has also been shown to be the leading cause of drug withdrawal for safety reasons post marketing. As shown by a survey conducted over the period 1961-1992 in France, Germany, the UK and the USA, 23 of 181 (13%) withdrawals from market for safety reasons were due to hepatotoxicity[30].

Recommended Actions

A better understanding of the mechanism(s) of liver toxicity as well as the underlying reasons why nonclinical studies fail to prevent compounds which produce serious human hepatotoxicity from proceeding in the clinic could result in developing a more predictive nonclinical testing strategy. Such technologies as those previously described under *In Vitro Cellular* Assays, Covalent Binding or Toxicogenomics/Proteomics should be considered for delineating the mechanisms of hepatotoxicities. Such methods could aid in identifying the molecular pathways of hepatotoxicity, covalent binding of compounds or metabolites to proteins and possibly neoantigens (produced from drug treatment) eliciting immune mediated toxicity. Their utility needs to be further assessed.

For compounds producing severe hepatotoxicities in humans, the nonclinical studies could be examined to determine if evidence of failed liver function occurred in any species, if safety margins were adequate between animal and human drug plasma concentrations or if differences in routes of drug metabolism existed between animals and humans. One can examine these cases for accumulation of reactive metabolites, potential for drug:drug interactions, or effects of disease states which would result in humans being more sensitive to hepatotoxicity than the animals models.

Summary

The purpose of nonclinical studies is to identify and assess the hepatotoxic potential of a drug in relevant models/studies covering the range of treatment regimens in clinical trials. Identification of hepatotoxicity is done primarily by clinical and morphologic pathology assessment measured at multiple time intervals in nonclinical studies. Appropriate selection and implementation of nonclinical studies will detect and characterize most hepatotoxicities. If hepatotoxicity occurs, the assessment must identify the changes and their magnitude, provide a NOEL/NOAEL, and determine a mechanism/pathogenesis for the lesion such that the clinician can formulate a development, risk and monitoring recommendation.

Nonclinical studies do not provide adequate assessment of all hepatotoxic liabilities in man. It is particularly important to understand unique factors responsible for human differences from laboratory animal models, and to modify models or testing strategies to account for such differences. Such factors include: genetic variability, lifestyle factors, formation of unique metabolites with hepatotoxic potential, immune mediated events, drug-drug or drug-food or drug-environment interactions, or endogenous or exogenous factors that modify or compromise organ or tissue function.

References

[1] Phol, L.R., Satoh, H., and Christ, D.D., et al: The immunologic and metabolic basis of drug hypersensitivities, Annu Rev Pharmacol, 28:367, 1988.

[2] Bird, G.L.A., Williams, R. Detection of antibodies to a halothane metabolite hapten in sera from patients with halothane-associated hepatitis, J Hepatol, 9:366, 1989.

[3] Pohl, L.R., Kenna, J.G., Satoh, H., et al., Neoantigens associated with halothane hepatitis, Drug Metab Rev, 20:203, 1989.

[4] Spielberg, S.P., Gordon, G.B., Blake, D.A., et al., Predisposition to phenytoin hepatotoxicity assessed in vitro, New Engl J Med, 305:722, 1981.

[5] Shear, N.H., Spielberg, S.P., Grant, D.M., et al., Differences in metabolism of sulfonamides predisposing to idiosyncratic toxicity, Ann Intern Med, 105:179, 1986.

[6] Beaune, P.H., Dansette, P.M., Mansuy, D., et al: Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome P450 that hydroxylates the drug. Proc Natl Acad Sci USA, 84:551, 1987.

[7] Klaassen CD, Eaton DL. Principle of toxicology. In: Amour MA, Doull J, Klaassen CD, eds. Casarett and Doull's Toxicology. The Basic Science of Poisons, 4th edn. New York: Pergamon Press; 1991: 12-49.

[8] Dean JH, Olson HM. The integration of investigative toxicology in the drug discovery process. Biol Cell 1993; 77: 3-8.

[9] Kapeghian JC, Traina VM. The role of experimental toxicology in safety evaluation; challenges facing the pharmaceutical industry. Med Res Rev 1990; 10: 271-80.

[10] Guillouzo A, Guguen-Guillouzo C Eds. Isolated and cultured hepatocytes. Paris: Editions INSERM and John Libbey Eurotext, 1986.

[11] Azri S, Gandolfi AJ, Brendel K. Precision-cut liver slices; an *in vitro* system for profiling potential hepatotoxicants. In Vitro Toxicol 1990; 3: 309-25.

[12] Fisher R, Brendel K, Hanzlik RP. Correlation of metabolism, covalent binding and toxicity for a series of bromobenzene derivatives using rat liver slices *in vitro*. Chem Biol Interact 1993; 88:191-208.

[13] Ballet F. Hepatotoxicity in drug development: detection, significance and solutions. J. Hepatology 1997; 26: (Suppl. 2): 26-36.

[14] Thibault N, Ballet F. Effect of bile acids on intra-cellular calcium in isolated rat hepatocyte couplets. Biochem Pharmacol 1993; 45: 289-93.

[15] Thibault N, Maurice M, Maratrat M, Cordier A, Feldmann G, Ballet F. Effect of tauroursodeoxycholate on actin filament alteration induced by cholestatic agents: a study in isolated rat hepatocytes couplets. J Hepatol 1993; 19: 367-76.

[16] Thibault N, Claude JR, Ballet F. Actin filament alteration as a potential marker for cholestasis: a study in isolated rat hepatocyte couplets. Toxicology 1992; 73: 269-79.

[17] Boelsterli UA. Specific targets of covalent drug-protein interactions in hepatocytes and their toxicological significance in drug-induced liver injury. Drug Metab Rev 1993; 25: 395-451.

[18] Monks TJ, Lau SS. Reactive intermediates and their toxicological significance. Toxicology 1988; 52: 1-53

[19] Nelson SD, Pearson PG. Covalent and non covalent interactions in acute lethal cell injury caused by chemicals. Annu Rev Pharmacol Toxicol 1990; 30: 169-95.

[20] Pumford NR, Myers TG, Davila JC, Highet RJ, Pohl LR. Immunochemical detection of liver protein adducts of the non-steroidal anti-inflammatory drug diclofenac. Chem Res Toxicol 1993; 6: 147-50.

[21] Bartolone JB, Sparks K, Cohen SD, Khairallah EA. Immunochemical detection of acetaminophen-bound liver proteins. Biochem Pharmacol 1987; 36: 1193-6.

[22] Bartolone JB, Birge RB, Sparks K, Cohen SD, Khairallah EA. Immunochemical analysis of acetaminophen covalent binding to proteins. Partial characterization of the major acetaminophen-binding liver proteins. Biochem Pharmacol 1988; 37: 4763.

[23] Lecoeur S, Bonierbalc E, Challine D, Gautier JC, Valadon P, Dansette PM, et al. Specificity of *in vitro* covalent binding of tienilic acid metabolites to human liver microsomes in relationship to the type of hepatotoxicity; comparison with two directly hepatotoxic drugs. Chem Res Toxicol 1994; 7: 434-42.

[24] Beaune PH, Dansette PM, Mansuy D, Kiffel L, Finck M, Amar C, et al. Human antiendoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis are directed against a human liver cytochrome that hydroxylates the drug. Proc Natl Acad Sci USA 1987; 84: 551-5.

[25] Nuwaysiv EF, Bittner M, Trent J, Barrett JC, Afshari CA. Microarrays and toxicology: Advent of Toxicogenomics. Mol Carcinog 1999; 24: 153-159.

[26] Lumley C. Clinical toxicity: could it have been predicted? Pre-marketing experience. In: Lumley CE, Walker SR, eds. Animal Toxicity Studies. Their Relevance for Man. Lancaster: Quay Publishing. 1990; 49-57.

[27] Olson H, Betton G, Robinson D, et al. Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. Reg Tox Pharm 2000; 32(1): 56-67.

[28] Zbinden G. Predictive value of animal studies in toxicology. Regul Toxicol Pharmacol 1991; 14: 67-77.

[29] Kornbrust DJ, McDonald JS, Chennekatu PP, Duchai DM, Stubbs RJ, Germershausen JI, et al. Toxicity of the HMG-coenzyme A reductase inhibitor, lovastatin, to rabbits. J Pharm Exp Ther 1989; 248: 498-505.

[30] Sprict-Pourra C, Auriche M. Drug Withdrawal from Sales, 2nd edn. Richmond: PJB Publications Ltd, 1994.

Appendix

Table 1: Parameters routinely implemented during nonclinical assessments for hepatotoxicity.

Parameter	Components	Collection Times	Advantages
Clinical Pathology	Clinical chemistry* · Hepatocellular leakage enzymes: ALT, AST · Cholestasis indicators: bilirubin, ALP, GGT · Function indicators: albumin, urea nitrogen · Metabolism indicators: electrolytes, total CO2, glucose, triglyceride, cholesterol · Additional standard chemistries Hemogram Leukogram Coagulogram Urinalysis	Intervals throughout all phases of study, but more frequently early in the study	· Certain hepatic changes can only be detected with clinical pathology parameters, especially clinical chemistry parameter data sets specific for liver. · Critical for determination of pathogenesis/mechanism of certain changes · Critical for determining severity and clinical implication of hepatic changes · Critical for monitoring onset, progression and reversibility. Some lesions are not monitorable by clinical pathology parameters. · Complimentary to histologic pathology
Morphologic Pathology	Histopathology Gross pathology Liver weight	Study termination (necropsy)	· Critical for identification of certain hepatic changes · Critical for determination of pathogenesis/mechanism of change · Complimentary to clinical pathology
Live phase	Clinical observations Body weight Food consumption	Throughout live phase	· In itself does not identify selected hepatic change, but does provide complimentary data and clinical consequence to hepatic changes
Enzyme induction	Cytochrome P450 subset measurement	Study termination	· Provides complimentary data for morphologic pathology findings · Critical for determining certain potential interactions in man

This document was prepared by an FDA Working Group in consultation with representatives of PhRMA and AASLD. It is not a guidance document. It does not contain recommendations to sponsors or applicants regarding particular actions they should take. In stead it is a concept paper that assesses the current state of knowledge, and the existing methodology for examining hepatotoxic events associated with pharmaceuticals. It is meant to provide a framework for discussion at a public workshop on drug-induced hepatotoxicity to be held February 12-13, 2001. As the knowledge evolves about this topic, the Agency may decide to develop guidance on how sponsors can better identify drugs that cause hepatotoxicity during the pre-clinical, clinical, and post-marketing periods.

* The clinical chemistry parameters listed are relevant examples, and are not inclusive of all parameters which may be measured.

Table 2. Findings in selected primary hepatotoxicity indicator parameters that are generally considered adverse.

Clinical pathology*	**Histologic pathology****
ALT: >3-5X increase	Hepatocellular degeneration
AST: >3-5X increase	Hepatocellular necrosis (including apoptosis)
GGT: >2X increase	Cholestasis
ALP: >3-5X increase	Inflammation
Bilirubin: absolute value >1 mg/dl	Fibrosis/cirrhosis
	Hepatocyte or Biliary proliferation

* Increases in clinical pathology parameters, even in the absence of histologic changes, are considered adverse, unless the pathogenesis indicates to the contrary.

** The occurrence of any histologic liver pathology change at or above the 'minimal to slight' level is considered adverse, unless the pathogenesis indicates to the contrary.

Table 3: Classification of liver changes by Process.

Classification	**Parameters used to detect**	
	Clinical pathology*	**Morphologic pathology**
· Degeneration/Necrosis	Yes (ALT, AST)	Yes
· Apoptosis	No	Yes
· Cholestasis	Yes (bilirubin, ALP, GGT)	Yes
· Adaptive (e.g., P450 enzyme induction, peroxisome proliferation)	No	Yes
· Proliferative	No	Yes
· Neoplastic	No	Yes
· Inflammation	Yes (leukocyte count, ALT, AST)	Yes
· Vascular	Yes (indirect changes)	Yes
· Metabolic	Yes (electrolytes, acid:base, ALT, AST)	Yes
· Accentuated normal function (e.g., increased lysosomal contents in Kupffer cells)	No	Yes

* Parameters listed are representative, and not all-inclusive.

Table 4. Classification of liver changes by Functional effect. Changes in functional effect are a result of the inciting process.

Classification	**Parameters used to detect**	
	Clinical pathology*	**Morphologic pathology**
· Hepatocellular injury	Yes (ALT, AST)	Yes
· Cholestasis	Yes (bilirubin, ALP, GGT)	Yes
· Altered Kupffer Cell activity	No	Suggestive
· Decreased functional hepatic mass	Yes (albumin, urea nitrogen, coagulation factors, bile acids)	Suggestive
· Acute hepatic failure	Yes (acid:base, electrolyte, ALT, AST)	Yes
· Altered hepatic blood flow	Yes (albumin, urea nitrogen, bile acids)	Suggestive

* Parameters listed are representative, and not all-inclusive.

Table 5: Animal Correlates for 31 Compounds Hepatotoxic in Humans

	(+) Rodent	**(-) Rodent**
(+) Non-Rodent	**8**	**8**
(-) Non-Rodent	**2**	**13**

Table 6: Phase of Clinical Development when Human Toxicity Determined

Terminated for Human Hepatotoxicity (n)	Phase I	Phase II	Phase III
Yes (15)[a]	7	8	
No (15)[b]	6	4	5

[a] Human hepatotoxicity predicted by animal model in 7 of 15 compounds terminated.

[b] Human hepatotoxicity predicted by animal model in 11 of 15 compounds not terminated.