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A comprehensive approach for drug safety assessment

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Abstract

A comprehensive, multidisciplinary approach is proposed here for the development of a drug with an acceptable safety profile. Key parameters to be considered for drug safety evaluation based on this comprehensive approach include the following: (1) Pharmacology: Possible toxicity due to drug–target interactions, including interactions with unintended molecular targets, or with molecular targets in unintended organs. (2) Chemistry: Chemical scaffolding and side-chains with safety concerns. (3) Toxicology: Toxicity in animals in vivo, and in relevant animal and human cells in culture. (4) Drug metabolism and pharmacokinetics: Safety concerns due to toxification or detoxification, organ distribution, clearance and pharmacokinetic drug–drug interactions. (5) Risk factors: Physiological, environmental and genetic factors that may enhance a patient's susceptibility. It is proposed that this integrated, multidisciplinary approach to safety evaluation may enhance the accuracy of the prediction of drug safety and thereby the efficiency of drug development.

Keywords: Paracelsus; Drug toxicity; Drug safety; Safety assessment; Risk factors; Drug development; Drug metabolism

1. Introduction

Classical toxicologists rely on Paracelsus' Principle [1]:

"All things are poison and nothing is without poison. Solely the dose determines that a thing is not a poison."

This principle of toxicology derived in the 15th century is the cornerstone of today's traditional practice of toxicology. Dose–response relationship is the most important data set from which safety is determined. For drugs, safety is estimated based on the therapeutic index, a ratio of the toxic dose to the dose required for efficacy. It is because of Paracelsus' Principle that toxicologists in general believe that safety can be estimated based on dose–response relationships without a need for mechanistic definition.

This empirical approach to safety evaluation is apparently not adequate, judging from the number of drugs with serious, sometimes fatal adverse effects, which have been erroneously concluded to have an acceptable toxicity profile in preclinical and clinical safety studies. It is proposed here that drug toxicity should be defined based not only on dose–response relationship, but also as a function of pharmacology, chemistry, metabolism, and environmental and genetic risk factors.

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2. A comprehensive approach to drug safety evaluation

The proposed comprehensive approach in drug safety evaluation is based on an integrated, multidisciplinary approach. This comprehensive understanding of drug safety should be applied towards all phases of drug discovery and development, from target identification through clinical trials.

The key scientific disciplines to be included in this comprehensive approach to drug safety evaluation include pharmacology, chemistry, drug metabolism and toxicology. A new discipline of risk factor identification is also proposed.

2.1. Pharmacology

As drugs are developed to be pharmacologically active, it is only logical that one should understand the safety concerns, if any, associated with the intended pharmacological effects. The toxicological ramification of the interaction of the drug candidate with the intended target in the target and nontarget tissues, and the likelihood of interactions with unintended targets, should be defined. This is especially important for a novel target with little preexisting clinical data. For instance, a novel target in the cell signaling pathways, which may have myriad cellular functions. Antagonists or agonists to a molecular target to cure a disease or to alleviate certain disease symptoms may lead to undesirable side effects due not only to the effects of the agent on the "normal" functions of the target but also the interactive cascade of events set off by engagement of the molecular pharmacology target, culminating in organ damage.

Anticancer drugs represent where pharmacological effects can be related to drug toxicity. Recently, novel targets have been proposed for anticancer drugs based on the novel discoveries in tumor cell and molecular biology including molecular controls of cell division, apoptosis, macromolecular processing, invasion and angiogenesis. As many of these molecular targets are also present in nontumor tissues, one needs to assure that the unintended toxicity in normal tissue is significantly less than that in the cancer cells, or at least develop a rationale for why the target remains an appropriate target even though toxicity to normal tissues is likely to occur (i.e. damage to normal tissue can be monitored and managed).

An interesting case of an unintended pharmacology-related adverse effect is associated with the biologic infliximab—a monoclonal antibody against tumor necrosis factor (TNF), indicated for the treatment of rheumatoid arthritis and Crohn's disease. The inhibitory effects of infliximab on macrophage activation are the mechanism for its desired anti-inflammatory effects. However, diminished macrophage activities cause an increased susceptibility of the patients towards infection. A warning was added to infliximab in 2001 [2] for the following reason as stated in a letter from the manufacturer to healthcare professionals:

". . .The Box Warning was added as a result of the occurrence of 84 cases of tuberculosis worldwide, during the period from August 24th, 1998, through June 30th, 2001 . . . An increased risk of infections associated with tumor necrosis factor blockade, is consistent with the known effects of TNF on macrophage activation and granuloma formation."

One should also anticipate possible drug–drug interactions based on pharmacological properties. A recent case of pharmacological drug–drug interaction is the interaction between sildenafil, a drug for erectile dysfunction. Sildenafil acts via the inhibition of cGMP-specific type 5 phosphodiesterase (PDE5). It also produces mild decreases in systolic and diastolic blood pressure and an array of minimal side effects, probably due to the inhibition of other types of phosphodiesterase. Drug interactions involving the concurrent use of sildenafil with nitrates and nitrites can produce profound hypotension leading to decreased coronary perfusion and myocardial infarction. A May 1998 letter from the drug manufacturer [3] warns that the drug is not to be co-administered with organic nitrates. This potentially fatal drug interaction also led to the withdrawal of several sildenafil-containing herbal medications from the market [4].

A conscientious effort to evaluate pharmacologically related adverse drug effects should allow one to avoid the selection of a problematic target and to identify management strategies early on to eliminate unexpected postmarketing adverse events.

Examples of pharmacology-related toxicological investigations are as follows:

1. Are there adverse effects as a result of the desired drug–target interactions?
2. Is the molecular target present in nontarget organs/tissue? If so, would there be adverse effects due to interactions with the pharmacological target in nontarget tissues?
3. Are pharmacological drug–drug interactions likely?
4. Is the pharmacological species a relevant model for human toxicology?

2.2. Chemistry

Many times during drug discovery, a project is abandoned because the major chemical structure (scaffolding) chosen has undesirable toxicity, which cannot be overcome via modification of the side-chains. It is therefore important to make sure that chemical structures with a high probability of success are chosen earlier in the program, especially when multiple chemical structures are found positive in the early screening process for efficacy.

One early approach is to evaluate whether the major chemical structure chosen has a history of safety-related problems. In silico approaches continue to be developed to correlate chemical structure with toxicity. As of this writing, it is generally believed that in silico approaches are adequate for the prediction of genotoxicity such as the Ames Salmonella/histidine-revision assay, but are not yet applicable for other types of toxicity (e.g. hepatotoxicity; cardiotoxicity) [5,6]. A promising approach is to perform in vitro toxicological assays early in drug discovery to allow the selection of the chemical structures with the least toxicological liabilities. Combined use of efficacy screens and in vitro toxicity screens allows one to evaluate whether the chemical structures important for efficacy (pharmacophores) can be distinguished from those responsible to toxicity (toxicophores).

Examples of chemistry-related toxicological questions are as follows:

1. Are there known adverse drug effects associated with the major chemical structure (scaffolding)?
2. Are there chemical side-chains with known toxicity (structural alerts for toxicity)?
3. Can the toxicophore be separated from the pharmacophore (using relevant in vitro or in vivo experimental models)?

2.3. Drug metabolism and pharmacokinetics

The relationship between drug metabolism and toxicity cannot be overemphasized. Metabolism-related toxicity is responsible for a number of adverse drug effects in the liver, the organ where first past drug metabolism occurs. Species-differences in drug metabolism represent a key reason for species-differences in drug toxicity. Pharmacokinetic drug–drug interaction, the effect of one drug on the metabolic clearance of a co-administered drug, is also a major mechanism of adverse drug effects. While organ-specific toxicity can be a function of metabolism in specific organs (e.g. liver, kidney), it also can be due to bioaccumulation (e.g. CNS toxicants).

An important development in drug metabolism is the general acceptance of human tissue-derived systems, especially human liver-derived systems such as liver microsomes and fresh and cryopreserved human hepatocytes, in the evaluation of human drug metabolism. Such studies include the evaluation of intestinal uptake, metabolic stability, metabolite identification and pharmacokinetic drug–drug interactions [7,8]. Drug metabolism data obtained with human in vitro system provides critical safety information such as the identification of toxification and detoxification pathways. Drug metabolism data are routinely used to guide the selection of the most “relevant” animal species for safety and pharmacology studies based on their similarities to human in metabolism. In vitro human systems allow one to develop human metabolism data before a drug candidate is administered to humans in vivo.

A consensus is being reached on drug metabolism properties, which appear to occur frequently in drugs with fatal idiosyncratic drug toxicity. These properties include the formation of reactive metabolites, enzyme induction, P450-related toxification pathways and propensity for drug–drug interactions [9–12]. These common properties are consistent with the proposed mechanisms of idiosyncratic drug toxicity and can be used to guide the elimination of drug candidates with high probability of causing idiosyncratic drug toxicity.

Examples of drug metabolism and pharmacokinetics-related toxicological questions are as follows:

1. Is the chemical entity biotransformed? If so, is it rendered more (toxification) or less (detoxification) toxic?
2. Are the human metabolites similar or different from the metabolites formed in laboratory animals? Which animal species is most like human?
3. How rapidly is the chemical entity cleared?
4. Is the chemical entity or its metabolites accumulated in specific organs?
5. Are pharmacokinetic drug–drug interactions likely to occur?

2.4. Toxicology

Well-designed toxicity studies are key to safety assessment. The commonly applied approach of a battery of genotoxicity assays, and studies with laboratory animals including acute, subchronic and chronic studies, developmental toxicity studies, and life-time carcinogenicity assays are invaluable in the evaluation of drug toxicity. The emphasis here is that the toxicity observed should be evaluated mechanistically to derive the most accurate prediction of human safety.

Although the definition of human safety is the ultimate goal, it is also important, during early phases of drug development, to predict animal toxicity that may occur during preclinical safety trials to allow one to design the most effective animal studies. A key in the use of laboratory animals is to use species that are most "relevant" to human, whenever possible. Key toxicity determinants that are different between the laboratory animals used and humans should be clearly defined to aid data interpretation.

An expert group recently concluded that human-based experimental systems are useful in aiding the prediction of human drug toxicity and that in vitro systems with primary cells, especially from human organs, serve as promising experimental systems for the evaluation of human-specific drug properties [7]. Examples of such assays are the use of human blood vessel endothelial cells in the evaluation of vascular toxicity [13], human hepatocytes in the evaluation of hepatotoxicity [7,8,14], and human kidney proximal tubule cells for nephrotoxicity [15]. A most recent development is an integrated multiple organ culture system which integrates cells from multiple organs for the evaluation of drug toxicity [16]. An advantage of in vitro experimental systems using primary cells, which retain human specific properties is that the results are likely to be relevant to human. A caveat of the use of in vitro systems is that care must be taken to avoid erroneous conclusions due to in vitro artifacts and the performance of experiments under physiologically irrelevant conditions (e.g. dose levels that would not be achievable in human in vivo), and to fully recognize the limitations of the in vitro system (e.g. the lack of blood circulation, excretion, multiple organ and tissue interactions (which may be improved via the use of the integrated co-culture system, idMOC [16]), and an intact immune system).

Toxicology studies should be performed using an investigative approach. Adverse effects should be further defined mechanistically, using endpoints and experimental systems, which may not be routine. In vitro approaches using primary cells from human or animal organs, high content assays such as genomics, proteomics, metabolomics, are examples of experimental tools for mechanistic studies. An example of an application of novel technologies is a recent study with troglitazone, a drug successfully marketed for the treatment of type II diabetes but was withdrawn due to its association with fatal liver toxicity. Troglitazone was found to induce a significantly higher number of gene expression changes for a battery of toxicologically relevant genes than the relatively nontoxic structure analogs rosiglitazone and pioglitazone [17]. Based on the differences between toxic and nontoxic compounds in their effects on gene expression, one can construct possible mechanisms of toxicity, which can be experimentally verified and applied towards the prediction of human effects. Additionally, the knowledge can be applied for the development of biomarkers of toxicity and the development of screening assays for specific toxic liability.

Examples of toxicological questions that are relevant to the prediction of human drug toxicity are listed below:

1. Is there toxicity observed with the chemical entity in vitro and in vivo?
2. Is the toxicity associated with the chemical scaffold or its side-chain?

3. Does drug metabolism or organ distribution contribute to the toxicity observed?
4. Would there be species differences in toxicity? If so, why?
5. What are the expected risk factors for toxicity?

2.5. Risk factor identification

While it is true that dose is key to toxicity, the dose that is toxic to different individuals may differ due to physiological, environmental and genetic factors. A dose that is nontoxic to a majority of the patient population may be fatal to an individual due to one or more of these factors (risk factors). A case in point, the analgesic acetaminophen is a safe drug but is known to cause fatal hepatotoxicity, especially in individuals who consumed alcohol. Alcohol has been identified as a risk factor for acetaminophen, presumably due to the induction of the metabolic "toxification" pathway (e.g. cytochrome P450 isoform 2E1) as well as the reduction of detoxifying protective cofactors (e.g. reduced glutathione) [18].

The risk factor approach in drug toxicity is implied in a recent proposed hypothesis for idiosyncratic drug toxicity, the Multiple Parameter Hypothesis, which states that the low frequency of idiosyncratic drug toxicity is due to concurrence of multiple independent events [6]. Based on the hypothesis, the probability for idiosyncratic drug toxicity (P_{idt}) is a product of the following independent probabilities: (1) exposure to the drug (e.g. dose; P_{exp}); (2) inherent biological properties of the drug due to its chemical structure (e.g. ability to form reactive metabolites; P_{chem}); (3) environmental risk factors (e.g. co-exposure to interacting foods or drugs; $P_{environ}$); and (4) host risk factors (e.g. genetic determinant for drug toxicity; disease conditions predisposing an individual to drug toxicity; P_{host}):

$$P_{idt} = P_{exp} P_{chem} P_{environ} P_{host}$$

A corollary of the Multiple Parameter Hypothesis is that there exist risk factors that can dramatically enhance a drug's toxic potential. Individuals in an environment at a specific point in time may have the "right" combination of risk factors that, if administered a drug with idiosyncratic toxic properties, would succumb to its toxicity.

$$\mathrm{Tox}_{individual} = f(D, \mathrm{Tox}_{inherent}, \mathrm{RF}_{total})$$

where $\mathrm{Tox}_{individual}$ is the toxicity of the drug in a particular patient at the specific time of administration (e.g. dose to cause liver failure); D the dose of the drug administered; $\mathrm{Tox}_{inherent}$ the inherent toxicity of the drug as related to the chemical structure; and RF_{total} represents a single risk factor as a result of all risk factors which can be physiological, environmental and genetic factors that the patient has or is subjected to that would enhance toxicity.

Definition of risk factors should be based on the mechanistic understanding of the key toxic pathways. For instance, if toxicity is due to the formation of toxic metabolites, one needs to define potential risks due to individual with enhanced toxification and/or reduced detoxification pathways as well as pharmacokinetic drug–drug interactions that may increase a patient's body burden of toxic metabolites.

As of this writing, risk factors associated with drug metabolism (e.g. induction of toxifying metabolic activities; reduction of detoxifying activities; polymorphism of metabolic enzymes; pharmacokinetic drug-drug interactions) probably are better defined than risk factors not associated with drug metabolism [19]. Current investigation of nonmetabolic risk factors for established drugs (e.g. inflammation, disease status) should help define risk factors of new drugs. There is evidence, for instance, that inflammation is a risk factor for drug induced liver failures [19,20].

A thorough understanding of risk factors based on known pharmacology, chemistry, drug metabolism, toxic mechanism, and patient characteristics will aid key decisions in drug development. An estimation of the probability of human populations with unfavorable risk factors (to allow a go/no-go decision), and the feasibility of the identification of at-risk populations (to allow safe administration of the drug), are information, which may be critical to the development of safe drugs.

Examples of questions regarding risk factors are listed here:

1. *Physiological risk factors*: Would specific age, gender, race and disease state enhance toxicity? Is the patient population known to be more susceptible to certain types of adverse drug effects (e.g. hepatotoxicity in the diabetic population)?
2. *Environmental risk factors*: Are there environmental conditions that can enhance toxicity? Are there co-administered drugs or foods that would lead to

toxicity due to either pharmacokinetic or pharmacological interactions?
3. *Genetic risk factors*: Are there genetic determinants of susceptibility to drug toxicity? For instance, is there known genetic polymorphism of the toxifying or detoxifying pathways (e.g. CYP2C9; uridine-dependent glucuronosyl transferase) in the human population?

2.6. Implementation

The proposed comprehensive approach allows one to assess drug safety intelligently and scientifically, and therefore should be an integral part of the drug discovery and development process, from target selection to clinical trials. Drug candidates conscientiously selected based on the implementation of this approach should have a higher probability of clinical success than drugs selected based mainly on efficacy alone.

This comprehensive approach is best practiced by a team with members with in depth knowledge of the multiple scientific disciplines described (pharmacology, chemistry, drug metabolism, pharmacokinetics, toxicology, genetics). An example of a Comprehensive Drug Safety Evaluation Team is one led by a toxicologist, with team members with expertise in pharmacology, chemistry, drug metabolism/pharmacokinetics, pathology, genetics and supplemented by other scientific disciplines (e.g. epidemiology, statistics, medicine) as needed.

Two major objections to the adoption of this comprehensive approach to drug safety evaluation are as follows:

1. *Complications with regulatory approval*: The old adage in regulatory toxicology is to present the regulatory agencies with the "cleanest" data package possible. Experimentations that may "complicate" the package are to be avoided at all costs. The price to pay for this approach is that data interpretations based purely on standardized, routine tests, without further investigative experimentations, may not allow one to accurately predict human drug safety. An investigative approach allows the presentation of scientific information and the rationale of the conclusion based on experimental data. It is argued here that via objective and scientific experimental approaches and data analysis, a drug candidate that is concluded to be safe to humans should rightly receive regulatory approval, and is in fact a more efficient approach than the current approach of a minimum data package and optimistically interpreting adverse data.
2. *Prolonged time and extra resources needed for drug development*: As it is difficult for toxicity to be clearly defined, this comprehensive approach may require investigations which may lead to further investigations, thereby requiring further investment in time and resources. As the ultimate goal is the selection of the best drug candidate so that a successful drug can be developed, it needs to be ensured that the team members are working at the highest efficiency to reach this goal. Delays will occur, but only for sound reasons. Additional investigative work early in drug development should be more than compensated by the subsequent decrease in failure rates in the clinic. The extra costs can easily be justified by the higher success rate in the clinical trials and the minimization of the incidence of withdrawal of marketed drugs due to unacceptable drug effects.

The underlying principle for the proposed approach is that the accuracy of drug safety can be enhanced via a multidisciplinary collaboration to allow a clear understanding of toxicity-related drug properties including pharmacology, chemistry, drug metabolism, toxicology and risk factors. Recent advances in informatics, high content assays such as genomics, proteomics, metabolomics, in vitro biochemical, molecular and cellular experimental systems, should aid this comprehensive approach to evaluate drug safety.

As drug toxicity is a key determinant of success, secondary only to efficacy, it should be an integral part of drug discovery and development. It is envisioned that the proposed integrated, multidisciplinary approach will enhance the efficiency of drug development via minimizing the probability of the development of drugs with unacceptable toxicity. Most of the studies outlined in this proposal are already being executed in most drug development programs—this approach simply place human drug toxicity as the major focus and driving force. Adaptation of this approach will no doubt involve more resources than the current "routine" approach, but it is expected that the end should justify the means—the minimization of costly

outcomes such as clinical failures and market withdrawal due to adverse drug properties should more than compensate for the extra initial expense.

Finally, the following modified version of the Paracelsus' Principle is proposed:

"While dose makes the poison, environmental, genetic, and physiological factors determines the dose that makes the poison for an individual"

The environmental factors include co-administered foods, drugs and environmental chemicals; genetic factors include drug metabolizing enzyme genes and various damage–repair genes; and physiological factors include size, age, gender and disease states.

Accurate prediction of human drug toxicity requires not just the analysis of dose–response relationship, but also a clear knowledge of the mechanism of toxicity and the corresponding risk factors.

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