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Screening for the potential of a drug candidate to cause idiosyncratic drug reactions

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Toxicity testing has been ineffective in the prediction of drug candidates that will be associated with a relatively high incidence of idiosyncratic drug reactions (IDRs). Circumstantial evidence suggests the involvement of reactive metabolites in the aetiology of these reactions and this has prompted several companies to screen drug candidates for the formation of such compounds. Most drugs form at least one reactive metabolite. To develop efficient prediction methods, a better understanding of the basic mechanisms involved is essential. This review highlights the current mechanistic hypotheses of IDRs and discusses future directions in the development of better predictive tests.

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Idiosyncratic drug reactions (IDRs) are a major impediment in drug development. Despite extensive animal testing and safety monitoring during clinical trials, 10.2% of drugs approved during 1975–2000 either had to be withdrawn from the market, or were given a 'black box' warning, as a result of adverse reactions that had not been predicted [1]. In addition, the development of an unknown number of drugs has been halted because evidence suggested that they might cause adverse reactions. The time and money expended trying to prevent adverse reactions, the loss of good drug candidates because of suspected problems, and the failures when drugs are withdrawn or restricted, all markedly increase the cost of drug development.

The problem of IDRs

Some of the adverse reactions responsible for withdrawals are a result of drug interactions or known pharmacological properties, such as prolongation of the cardiac QT interval; such adverse reactions are more easily predicted today than in the past [2]. More problematic are the truly idiosyncratic reactions that do not involve the known pharmacological properties of the drug. Such reactions have characteristics suggesting that they are immune-mediated: there is a delay of a week or more before onset of the reaction and there is no simple dose–response relationship [3]. Examples include troglitazone-induced hepatitis [4], felbamate-induced aplastic anaemia [5] and lamotrigine-induced skin rashes [6]. In the past, IDRs were generally ignored by the industry because of the unpredictable nature of these reactions. However, the magnitude of the problem and the increased cost of drug development make it difficult to justify this strategy, and several pharmaceutical companies have set up programmes to eliminate potentially dangerous drug candidates in the early stages of development [7]. Current hypotheses for the mechanisms of IDRs and strategies to screen drug candidates for their potential to cause such reactions are highlighted here.

Postulated mechanisms

Hapten hypothesis

The general characteristics of most IDRs suggest that they are mediated by the immune system. If this is the case, the idiosyncratic nature of these reactions is easier to rationalize because some people are allergic to particular compounds, whereas others are not. There are some examples, such as allergic reactions to penicillin and halothane hepatitis, in which clear involvement of the immune system has been demonstrated; however, in most cases, involvement of the immune system is only inferred.

Another aspect of IDRs that is generally accepted is the involvement of reactive

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metabolites [8,9]. With the exception of compounds like penicillin (here, the reactive moiety is the parent drug and the formation of a reactive metabolite is not required), this is also based on circumstantial evidence. The 'hapten hypothesis' has been used to link the reactive metabolites with immune-mediated IDRs. It proposes that modification of a protein by a reactive metabolite creates a 'foreign' protein, which, in some cases, leads to an immune-mediated adverse reaction [10].

Danger hypothesis

There are shortcomings with the hapten hypothesis. For example, foreign proteins do not usually lead to a significant immune response in the absence of an adjuvant. This is because an immune response requires a second signal (signal two), controlled by the expression of co-stimulatory molecules on antigen-presenting cells. There are several stimuli that can act as adjuvants to upregulate the co-stimulatory molecules, including specific molecules associated with pathogens (exogenous stimuli) [11] and cell damage or necrotic cell death (endogenous stimuli) [12]. Furthermore, there does not appear to be any mechanism by which antigen-presenting cells, which control the level of signal two, can differentiate 'foreign' from 'self'. This concept forms the basis of the 'danger hypothesis', which proposes that danger to an organism, not 'foreignness', is the major driving force behind the induction of an immune response [13]. Therefore, another possible link between the formation of a reactive metabolite and the risk of a drug inducing an IDR is suggested; the reactive metabolite could damage cells leading to a 'danger signal' and upregulation of signal two [14,15].

It is more difficult to induce an immune response agaist normal 'self' proteins because most of the T cells with the strongest affinity for self proteins are eliminated in the thymus. However, some autoreactive T cells survive, and autoimmune reactions (i.e. immune responses against self proteins) do occur [10]. Several drugs that cause idiosyncratic liver toxicity, such as isoniazid and halothane, are associated with transient elevations of transaminases [16], probably representing direct toxicity and, hence, a danger signal. It has been suggested that the adverse reactions associated with some other drugs, such as troglitazone, are due to effects on the mitochondria [17]. Such effects would also be expected to generate a danger signal. Thus, there could be a relationship between direct cytotoxicity and the risk that a drug will cause a significant incidence of IDRs. However, most drugs that are associated with IDRs do not cause such overt evidence of toxicity and more-subtle types of cell stress are probably more common.

P-I hypothesis

The P-I (pharmacological interaction) hypothesis provides a new theory [18]. It is based on the observation that some T cells from patients with a history of an IDR to a specific drug proliferate on exposure to this drug in the absence of metabolism that could form a reactive metabolite. This suggests that the T cells recognize the parent drug, rather than reactive-metabolite-modified peptides, as proposed by the hapten hypothesis. This, in turn, implies that covalent binding of reactive metabolites might not be required for the induction of an IDR, and that the involvement of reactive metabolites could be a direct result the induction of a danger signal. However, to date, there is no evidence that this type of direct interaction can initiate an IDR.

A fundamental question is whether the formation of reactive metabolites is necessary for a drug to cause idiosyncratic drug reactions and, if so, what role do they play? Do they modify proteins to make them immunogenic (hapten hypothesis), do they cause cell damage that stimulates the immune system (danger hypothesis), or are both roles important?

Types of reactive metabolites

Quinones and related structures

There are many different types of reactive metabolites that can be formed by drugs, a detailed description of which can be found elsewhere [19]. In general, reactive metabolites are either electrophiles (i.e. electron deficient) or free radicals. Electrophiles usually react with biological nucleophiles, such as glutathione or nucleophilic groups on proteins, such as sulfhydryl or amino groups. A common type of reactive metabolite are quinine compounds and related quinone imines and quinone methides. These can be formed by oxidation whenever there are -OH groups para or ortho to one another on an aromatic ring. The quinone imine is formed when one of the -OH groups is replaced by an amino group, and the quinone methide is formed when the one of the -OH groups is replaced by a methylene group [20]. Even if the parent drug does not have such an arrangement, oxidation of an aromatic ring is a common metabolic pathway, adding an -OH to the aromatic ring, often para to an existing hydroxy, amino or methylene group. This type of reactive metabolite is, therefore relatively common.

Aromatic amines, nitro groups and hydrazines

Aromatic amines are less commonly present in drugs, but they are usually associated with a significant incidence of IDRs [21]. Aromatic amines are often oxidized to reactive nitroso groups. The same reactive metabolite is formed by the reduction of a nitro group, and drugs that contain

aromatic nitro groups are associated with a high incidence of IDRs. Hydrazines are also oxidized to reactive species and have been linked with a high incidence of IDRs.

Acyl glucuronides and epoxides

Carboxylic acids can be activated by forming acyl glucuronides and/or CoA (coenzyme A) esters [22], although many drugs that contain carboxylic acids, such as the fibrates, are not associated with a high incidence of IDRs [23]. Likewise, arene oxides appear to be common intermediates in the oxidation of aromatic rings, but their involvement in IDRs is unclear [24]. In fact, most drugs form more than one reactive metabolite and hence, it is difficult to prove which, if any, is actually responsible for a specific IDR.

Covalent binding versus oxidative stress

The examples of reactive metabolites described here can covalently bind to proteins and act as haptens. Some of them, such as aryl amines, nitro groups and quinines, can also undergo redox cycling and induce oxidative stress, which could lead to a danger signal [21]. By contrast, free radicals usually abstract a hydrogen atom from other molecules, such as lipids or glutathione, and can also cause oxidative stress; however, they rarely form adducts with proteins. If reactive metabolites are responsible for most IDRs, it should be possible to eliminate 'bad' drug candidates by screening them for their ability to form reactive metabolites; several pharmaceutical companies have instituted programmes to do just this. However, it is also important to elucidate which characteristics of the reactive metabolites are responsible for IDRs.

Methods of screening for reactive metabolites

Avoiding suspect functional groups

The first step in avoiding reactive metabolites is to avoid, whenever possible, functional groups, such as aryl amines, that are known to readily form reactive metabolites [21]. In addition, metabolic pathways of the drug that would be expected to form reactive metabolites can often be anticipated. Early metabolic screens might produce metabolites that point to the formation of reactive intermediates. An obvious example of this is the finding of a glutathione conjugate, which is usually formed from an electrophilic metabolite or intermediate [25]. However, this is not the only type of metabolite that should provide a warning; for example, during a study of the oxidation of vesnarinone, we found that a major product represented the deamination of a tertiary aryl amine [26]. The only reasonable pathway leading to this stable metabolite was a reactive iminium ion, which we were able to trap with glutathione. Thus, many reactive metabolites can be avoided simply by understanding their potential implications and by keeping the issue in mind at each step in the drug development process. These can be considered as 'passive' screens because no additional experiments to search for reactive metabolites are required.

Screening for glutathione conjugates

A relatively easy 'active' screen for reactive metabolites is a search for glutathione conjugates. Glutathione is a major scavenger of reactive metabolites; therefore, a screen for the formation of a glutathione conjugates will pick up a significant fraction of reactive metabolites formed from a drug. This is relatively easy to do by LC–MS because glutathione conjugates have a characteristic fragment ion that can be detected by a neutral loss scan at m/z 129. These studies are best done *in vitro* (e.g. in hepatic microsomes or hepatocytes) because glutathione conjugates are often further metabolized *in vivo*. Unfortunately, this method does not detect all reactive metabolites, either because the conjugate is not sufficiently stable, for example where glutathione conjugates form from acyl glucuronides and/or CoA esters, or because the reactive intermediate reacts with other nucleophiles, usually nitrogen nucleophiles.

Some metabolites are so reactive that they often react with the first molecule they come into contact with. This will usually be the enzyme that formed the reactive metabolite and, hence, irreversible inhibition of P450 can be another important clue to the formation of a reactive metabolite. This inhibition can also lead to drug–drug interactions, which are clearly an undesirable trait. Glutathione conjugates (or their corresponding mercapturic acids) are not always observed *in vivo*, probably because they are transported into bile and destroyed by gut bacteria. This is more likely with high-molecular-weight drugs.

Covalent binding

An important method for detecting, as well as quantifying, the formation of a reactive metabolite is the use of radiolabelling to measure irreversible binding. This is obviously limited by the availability of radiolabelled drug and, therefore, is not often used as an initial screen. This method can give falsely low indications of covalent binding if the system used does not contain the enzyme responsible for the formation of the major reactive metabolite. For example, vesnarinone, a drug associated with the induction of agranulocytosis, is rapidly oxidized to a reactive metabolite by myeloperoxidase/hydrogen peroxide, the major oxidation system in neutrophils (the target cell

type in agranulocytosis) [26]. However, less of this reactive metabolite is formed by P450. Therefore, if the only system used to detect covalent binding involved liver microsomes or hepatocytes, it would be concluded that little reactive metabolite is formed.

The radiolabelling method can also give a falsely high prediction of covalent binding with an *in vitro* system because, *in vivo*, many reactive metabolites are efficiently scavenged by glutathione or other detoxication system and cause little damage. Therefore, the use of hepatic microsomes to determine covalent binding can lead to a falsely high estimation of the *in vivo* covalent binding of a drug.

Using a combination of methods and a variety of tissues

Considering the issues mentioned previously, the best method to detect covalent binding is probably the application of a combination of systems. Hepatocytes have the advantage that they contain a combination of enzymes. Furthermore, human hepatocytes are available for such use, making it possible to determine any differences between humans and other species in the formation of reactive metabolites. However, as already indicated, the liver is not the only target for IDRs and many reactive metabolites are too reactive to reach sites distant from where they are formed. Therefore, representative cells from the bone marrow and skin should be included because these tissues are frequent targets of IDRs.

Neutrophils have the greatest potential of bone marrow cells to form reactive metabolites because they generate hydrogen peroxide and contain myeloperoxidase, which, in combination, can readily oxidize many drugs to reactive metabolites. The skin contains fewer enzymes that can form reactive metabolites, but there are examples where metabolic activation can occur here [27].

Difficulties in data interpretation

If several different screening methods were used in several different tissues to screen all drug candidates, and all candidates that showed any evidence of bioactivation were eliminated from further development, few drugs would ever be developed. Almost all drugs form at least one reactive metabolite and many drugs that form reactive metabolites are relatively safe. How, then, can potentially dangerous drug candidates be differentiated from those that are likely to be safe? One obvious criterion is the amount of reactive metabolite that is formed. Although it is relatively easy to quantify covalent binding *in vitro* and only a little more difficult in animals, it is virtually impossible to quantify covalent binding in the target organs of toxicity in humans. Consequently, we have no data from which to examine the correlation between the amount of covalent binding and the risk of IDRs in humans. On an empirical basis, drugs administered at a total dose of 10 mg day^{-1} are unlikely to be associated with a high incidence of IDRs and, conversely, drugs administered at high doses, such as felbamate and procainamide, tend to be associated with a higher risk of IDRs [14]. The notion that IDRs are dose-independent is erroneous. Taken to the extreme, one molecule of a drug will not cause an IDR in anyone. However, most patients will not experience an IDR at any dose of a drug, and thus, the dose–response relationship is complex. Taken to the level of covalent binding, a level of up to 50 pM mg^{-1} of microsomal protein has been proposed by Lance Pohl, an expert in the field, as a safe upper limit of covalent binding, and this is currently used as a rough guide at Merck (http:// www.merck.com) [28].

Although there are no firm data, it is probable that binding of drug to some types of protein is more likely to lead to an IDR than binding to other types of proteins. Furthermore, as discussed, causing cell damage or creating a danger signal might be more important than covalent binding; therefore, reactive metabolites, such as free radicals, could cause a high risk of IDRs without any significant covalent binding.

Risk versus benefit

Screening for reactive metabolites would probably significantly decrease the risk of IDRs from new drugs, although this is yet to be demonstrated. However, until we have a better understanding of the precise role of reactive metabolites in the mechanism of IDRs, such screening is likely to result in a high level of false positives and false negatives. Therefore, the results of these tests must be considered in the context of other factors when making a final decision on whether to proceed with development of the drug. These include factors such as the indication for the drug (e.g. treatment of a life-threatening illness), comparison with other drugs for the same indication, and the ease by which the design of a drug can be altered so that a particular reactive metabolite is no longer formed.

Future directions

The development of better screening tests to predict IDR potential would clearly have a major impact on drug development (potential screening methods are listed in Box 1). However, a fundamental question remains: what is the role of reactive metabolites? Various other screens have been proposed to answer this question and are currently being used although they lack validation – many involve simple *in vitro* cytotoxicity assays – but results thus far are

Box 1. Potential methods to screen drugs for their potential to cause idiosyncratic drug reactions

1. Screening for the formation of reactive metabolites
This is relatively easy to do and is likely to lead to safer drugs but has not been demonstrated. Furthermore, it is unlikely that all reactive metabolites are equal; until we understand the role of reactive metabolites in IDRs, it will be difficult to interpret the data.

2. Using markers of cell stress to screen drug candidates
If the 'danger hypothesis' is correct, there should be patterns of cell stress that predict the potential of a drug candidate to cause IDRs. Examples of potential markers include induction of glutathione transferases and quinone reductase. There is preliminary evidence to support this hypothesis but it is too early to be certain. The complexity of this system and, particularly, of the immune system, can not be duplicated *in vitro* and, thus, *in vivo* screening would be necessary.

3. Screening for specific markers of cell toxicity
Although standard markers of toxicity have not been accurate in predicting IDR potential, new markers, such as mitochondrial function, might be more specific. Further research is required to test this idea.

4. Using gene chips to determine patterns of gene expression that are associated with IDR potential
This strategy has the advantage that it does not require prior knowledge of mechanisms and can examine many different genes. The disadvantage is that gene chips are relatively insensitive to small changes, which could be a particular problem when studying complex tissues, where the changes of interest will only occur in a subset of the cells and could, therefore, be obscured. Different drugs will probably cause different patterns, and interpretation of many complex sets of data will be a lengthy process. However, such data might point to more specific biological markers that predict IDR potential.

not encouraging. High concentrations are used that probably cause changes very different from those that occur in a clinical context. If standard *in vivo* toxicity tests with various end points, such as histology and liver transaminases, have not been accurate at predicting IDR potential, it seems unlikely that using cell death in a cell culture will be more predictive. The complex *in vivo* environment that enables a balance between formation and detoxication of reactive metabolites is virtually impossible to mimic in a test tube. Furthermore, cell death in culture as an end point cannot reflect the types of cell stress that are more likely to contribute to the induction of an immune response *in vivo*.

Identifying markers

If the danger hypothesis is correct, there will be biological markers of cell stress that correlate with IDR potential. There is evidence that drugs associated with IDRs cause an upregulation of protective enzymes *in vivo*, but more studies are required before we can be confident that such changes are predictive; different drugs will probably be associated with different patterns, thus complicating the prediction process. In addition, the response might vary between species; to be certain that a drug candidate did not cause cell stress, it would be prudent to repeat the studies in Phase I trials. Other markers of specific types of cell toxicity that have not been used in the past, such as mitochondrial toxicity, might also predict IDR potential.

Gene chips could help to find the best markers for risk prediction and this is most likely to be successful in relatively homogeneous tissues, such as the liver. The skin and bone marrow comprise a more complex mixture of cells and thus, will be more difficult to study because the crucial changes in gene expression will occur in only a subset of cells. For example, a search for specific changes in gene expression using the whole spleen produced negative results, but when specific cell subsets were isolated, major changes in gene expression were evident (J. Uetrecht *et al.*, unpublished). Although speculative at present, the development of biological markers that predict the risk of a drug being associated with a high incidence of IDRs remains a realistic goal.

Understanding mechanisms

For the reasons discussed here, the development of better screening tests for the potential of a drug candidate to cause IDRs depends on a better understanding of the mechanisms of such reactions. These reactions are too complex to duplicate in a test tube, and their idiosyncratic nature precludes prospective clinical studies. Although animal models are very difficult to find because IDRs are just as idiosyncratic in animals as they are in humans, they are an important tool for mechanistic studies. For example, a novel animal model of nevirapine-induced skin rash in rats has significant potential in such studies [29]. However, not all IDRs will be identical and hence, it is important that we have more than one animal model. It now seems likely that the response of most patients to a drug that is associated with a relatively high incidence of IDRs is immune tolerance [30] and therefore, advances in our basic understanding of immune tolerance could also result

in a better understanding of IDRs. In fact, if we knew how to overcome immune tolerance, development of animal models of IDRs might be simple.

Genetics as a tool

Genetic factors appear to have a crucial role in the induction of IDRs. It is widely believed that 'blockbuster' drugs are a thing of the past and, in the future, we might assess patient genotype to determine which drugs will be effective and safe for each individual patient. However, this looks unlikely to happen soon. One obvious reason is that many patients would have to experience a serious IDR to a new drug before there would be sufficient patient samples on which to perform the necessary genetic studies. Furthermore, unlike the genetics of a pharmacological response, which is likely to be under the control of a few genes, the complex nature of IDRs implies that many genes (hundreds if not thousands) will have a role. There might be cases in which the situation is not so complex, but these will probably be the exception, rather than the rule.

The best data collected so far is for the association of abacavir adverse reactions with specific HLA-encoding alleles [31], but this finding is not considered sufficiently predictive to be used clinically at present. Scepticism remains that genetic studies will, in the near future, allow the prediction of which individuals will have an IDR to a specific drug, but, as a research tool, genetic studies have the potential to contribute significantly to our understanding of the mechanisms involved.

Next steps

Although IDRs are extremely complex and difficult to study their implications in drug safety make them impossible to ignore. To predict accurately which drugs are likely to cause such reactions, we need to improve our understanding of the basic processes. However, it is likely that it is already possible to make drugs safer, despite a significant number of false positives and negatives, by avoiding drugs that form substantial amounts of reactive metabolites.

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