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In vitro and animal models of drug-induced blood dyscrasias

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Abstract

Drug-induced blood dyscrasias can be either acute and predictable or delayed and unpredictable (idiosyncratic). The predictable toxicity is relatively easy to reproduce with in vitro models, although they may not work for drugs that require bioactivation. It is very unlikely that idiosyncratic blood dyscrasias can be modeled in vitro, although some drugs (or their reactive metabolites) that cause idiosyncratic reaction are toxic to bone marrow cells in vitro. Although the mechanisms of idiosyncratic reactions are poorly understood, there is evidence that most are due to reactive metabolites and some are immune-mediated. Therefore screening drugs for their bioactivation by myeloperoxidase, the major oxidative enzyme in bone marrow, may provide some measure of the risk that a drug will cause blood dyscrasias. Several examples of drug-induced idiosyncratic agranulocytosis, aplastic anemia and thrombocytopenia are presented, but better in vivo models are clearly needed to gain a clearer understanding of these adverse reactions.

Keywords: Idiosyncratic drug reactions; Reactive metabolites; Agranulocytosis; Aplastic anemia; Thrombocytopenia

1. Introduction

Drug-induced blood dyscrasias are a significant cause of patient morbidity and mortality. They can effect any type of blood cell and common examples include agranulocytosis, which is a loss of granulocytes (neutrophil count of less than 500 cells/μl blood) leading to an increased risk of infections; thrombocytopenia, which is a decrease in the number of platelets leading to an increased risk of bleeding; and aplastic anemia in which all of the bone marrow-derived blood cells are decreased and most of the normal bone marrow cells are replaced by fat. Drug-induced blood dyscrasias can either be acute and predictable, such as the bone marrow toxicity associated with most anticancer agents, or delayed and idiosyncratic, such as clozapine-induced agranulocytosis. Our understanding of the basic mechanisms of such reactions as well as our ability to predict which drug candidates will cause them is quite limited; therefore, we need to have model systems to study mechanisms as well as systems to screen drug candidates for their potential to cause this type of toxicity.

2. Acute, predictable bone marrow toxicity

If an agent causes acute, predictable bone marrow toxicity at therapeutic doses it is unlikely to be suitable as a drug. One exception is anticancer agents where the dose-limiting toxicity is usually bone marrow toxicity. The goal of anticancer drugs is to kill cancer cells, but most agents are not very selective and bone marrow cells are especially vulnerable presumably because of their rapid rate of cell turnover. Therefore, even though the precise mechanism by which anticancer agents exert their therapeutic effects is often poorly understood, this mechanism is usually closely related to the mechanism by which they cause bone marrow toxicity. Some anticancer drugs, such as methotrexate and azathioprine (a precursor of 6-mercaptopurine) are also used as immunosuppressants, but at doses that do not cause significant bone marrow toxicity in most patients.

The bone marrow toxicity associated with anticancer drugs is relatively easy to predict based on their pharmacological effects and is usually manifested in animal toxicity studies; however, there can be a significant difference in the sensitivity between different animal species and humans. Pessina et al. have cultured human umbilical cord blood cells under conditions that produce granulocyte/macrophage colony forming units (CFU-GM) and they used this in vitro system to study bone marrow toxicity (Pessina et al., 2003). They found a good correlation between IC90 in this system and the maximum tolerated dose

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(mg/m^2/dose where m^2 is the surface area of the patient) in humans. It is somewhat surprising that they used a correlation between concentration in the in vitro system and dose in humans because there can be large differences between drugs with respect to volume of distribution and clearance leading to a poor correlation between dose and blood concentration. It is also surprising that cyclophosphamide fitted reasonably well because it requires prior metabolic activation by cytochrome P450, and the levels of this enzyme are likely to be low in this in vitro system. Although this in vitro system may be of use to screen agents that are expected to have direct bone marrow toxicity, it is unlikely to be of much use as a general screen of drug candidates because most drug candidates are unlikely to cause direct bone marrow toxicity at therapeutic concentrations, and therefore almost all candidates should be negative. The few drug candidates that unexpectedly cause direct bone marrow toxicity are likely to be detected in animal toxicity studies, which are likely to be better at detecting unexpected toxicity because an in vitro system does not have the full range of enzymes that might be required for bioactivation. As discussed in the next section, this in vitro system is very unlikely to detect drugs that cause idiosyncratic bone marrow toxicity and the authors do not claim that it would.

3. Delayed, idiosyncratic blood dyscrasias

Idiosyncratic drug reactions can affect almost any organ, and blood cells are a common target. The unpredictable nature of idiosyncratic reactions makes them difficult to deal with clinically, and it also makes it difficult to study their mechanism. The emphasis of this review will be on this type of reaction. Although little is known with certainty about the mechanism of most idiosyncratic drug reactions, there is a large amount of circumstantial evidence to point to the involvement of reactive metabolites (Pirmohamed et al., 1996; Uetrecht, 1999). In addition, characteristics like the delay between starting treatment and the onset of the reaction with a shortened delay on re-exposure suggest that they are immune-mediated (Park et al., 1998; Uetrecht, 1999). Even the unpredictable nature suggests involvement of the immune system because we are accustomed to some people being “allergic” to an agent while most people are not, and no other basis for the idiosyncratic nature has been found for most idiosyncratic reactions. However, with very few exceptions, there is no conclusive evidence that idiosyncratic reactions are immune-mediated, and it is likely that some are not.

If most idiosyncratic drug reactions are due to reactive metabolites then screening drug candidates for the formation of reactive metabolites might be a way to eliminate high-risk candidates (Uetrecht, 2000). In fact, this type of screening is performed by several pharmaceutical companies and the practice is becoming more common (Evans et al., 2004). However, the most common screening methods are not optimal for detecting drugs that will cause blood dyscrasias. Most reactive metabolites are too reactive to reach sites distant from where they are formed, and therefore reactive metabolites that cause blood dyscrasias are most likely formed by blood cells. Most reactive metabolite screens utilize bioactivation systems based on the liver, such as hepatic microsomes or hepatocytes, because the liver has the highest concentration of most metabolic enzymes. However, blood and bone marrow cells can also bioactivated some drugs (Uetrecht, 1992), and some drugs, such as vesnarinone discussed below, are more readily bioactivated by blood cells than by the liver. To our knowledge, blood cells are rarely used to screen for the formation of reactive metabolites. Neutrophils, in particular, have high levels of NADPH oxidase and myeloperoxidase, which together can oxidize many drugs, either directly or by producing hypochlorite, which can also oxidize drugs as discussed below (Uetrecht, 1992).

As with other forms of idiosyncratic drug reactions there are generally no in vitro tests that would predict the risk that a drug would cause idiosyncratic blood dyscrasias. This is not surprising because it would be impossible to reproduce the complex combination of factors that are involved in a test tube, especially if the reaction is immune-mediated. A workshop was set up to make recommendations on the use of in vitro systems for evaluating haematotoxicity (Gribaldo et al., 1996). Several different assays were described using different types of cells, but again there is no evidence that such assays predict the risk of idiosyncratic blood dyscrasias. The issue of metabolic activation was also discussed and it was proposed that S9 liver fractions or liver microsomes be added to the incubations. A more recent paper reported the in vitro toxicity of several drugs known to cause idiosyncratic bone marrow dyscrasias, such as carbamazepine, phenylbutazone and indomethacin but the concentrations required to cause inhibition were all above 200 μM, which is orders of magnitude above the therapeutic concentration (Negro et al., 2001). They then discussed the advantages and disadvantages of different metabolic systems that can be added to the in vitro assays that might improve the accuracy of the assay, but there were no data to indicate that such metabolic systems lead to a reliable assay.

It is possible that there could be biomarkers that would predict the risk that a drug would cause idiosyncratic blood dyscrasias (Uetrecht, 2000), and it is likely that pharmaceutical companies are using microarrays to search for such biomarkers; however, it may be more difficult for bone marrow which is composed of a more complex mixture of cells than some other organs such as the liver and so changes in one minor cell type may be obscured by the lack of change in the majority of cells.

In vitro assays have also been used to study the mechanisms of idiosyncratic reactions, such as agranulocytosis, and in some cases, the toxicity of the drug is greater to cells from affected patients than to cells from normal controls (Parent-Massin et al., 1993). However, such studies have not added much to our basic understanding of the mechanism involved. Such studies may provide some diagnostic clues to the specific agent responsible for an adverse reaction in patients.

There are also no good animal models of idiosyncratic blood dyscrasias. This is a bit surprising; however, even though such reactions can occur in animals they are also idiosyncratic in animals, and a model is not very useful if the reaction only occurs in a very small fraction of the animals treated. It might