BIOINFORMATICS, METABOLOMICS AND IDIOSYNCRATIC DRUG TOXICITY: NEW HOPE FOR OLD PROBLEMS?
J. Steven Leeder
Section of Developmental Pharmacology and Experimental Therapeutics, Children’s Mercy Hospital, Kansas City, MO 64108, USA

Introduction
Severe adverse drug reactions (ADRs) remain an ever present concern for all clinicians who utilize medications in children and adults. The term “idiosyncratic drug toxicity” generally refers to relatively rare, but potentially life-threatening events such as aplastic anemia, toxic epidermal necrolysis or fulminant hepatotoxicity in which the determinants of susceptibility (largely unknown) are thought to be unique to the individual experiencing the adverse event. Some drug-induced ADRs are referred to as “hypersensitivity” reactions and represent idiosyncratic reactions in which the immune system appears to be involved. Nevertheless, drug bioactivation to chemically reactive metabolites is widely considered to be a critical step in initiating downstream pathogenic events [1-3]. As a result, genetic variation in the enzymes responsible for drug bioactivation and/or detoxification is thought to be a key determinant of risk for developing an idiosyncratic ADR [4]. However, the response of a biological system to xenobiotic exposure is complex (e.g., clinical manifestations of idiosyncratic drug toxicity), and it can be expected that the pharmacogenetic determinants of individual susceptibility to idiosyncratic toxicity will involve more genes than those involved in drug bioactivation and detoxification. The challenge of the “genome era” is to develop and apply new tools to improve our understanding of complex phenotypes such as idiosyncratic drug reactions.

Establishing the Link Between Drug Bioactivation and Immune Responses
The term “anticonvulsant hypersensitivity syndrome” was introduced in 1988 to describe the triad of fever, rash and lymphadenopathy with or without hepatotoxicity that generally occurs within the first two to three weeks of treatment with the aromatic anticonvulsants carbamazepine (CBZ), phenytoin or phenobarbital [5]. We have observed anti-microsomal antibodies in the sera of a subset of anticonvulsant hypersensitivity patients using rodent liver microsomes [6, 7], and subsequently identified CYP3A1 and CYP3A2 as the predominant antigens. Epitope-mapping studies revealed a common epitope within the K-helix region of CYP3A1 [8], and the importance of the K-helix as an antigenic determinant has been confirmed by others who have mapped conformational epitopes on CYP2C9 (tienilic acid hepatitis) and CYP1A2 (dihydralazine hepatitis) [9, 10]. Rat CYP isoforms damaged in the bioactivation process undergo accelerated proteosomal degradation by ubiquitin-dependent proteolytic mechanisms [11, 12] and CYP3A4 inactivation by cumene hydroperoxide has been reported to be accompanied by heme modification of amino acid residues localized to the K-helix and the proximal L-helix/conserved cysteine domain [13].

Using CBZ as a model substrate, we have pursued the hypothesis that the ultimate product of the bioactivation process is an irreversibly damaged protein, which is targeted for enhanced proteolysis by pathways corresponding to MHC class I antigen processing and presentation to cytotoxic T-lymphocytes. The first step in this process was to identify to CBZ metabolite(s) that might be more proximal substrates to the actual protein-reactive metabolite and investigations to date have focused on the formation of 2-hydroxy CBZ (2-OH CBZ) and 3-hydroxy CBZ (3-OH CBZ), metabolites that could lead eventually toward formation of reactive iminoquinone and o-quinone metabolites, respectively (Fig. 1).

Potential Role for Metabolomics
While carbamazepine 10,11 epoxide is the major CBZ metabolite produced by human liver microsomes, ring-hydroxylated metabolites (2-OH-CBZ and 3-OH-CBZ) are also generated. 3-OH-CBZ is formed by human liver microsomes at rates >25 times those of 2-OH-CBZ. Both 2-OH-CBZ and 3-OH-CBZ formation appear to conform to monophasic Michaelis-Menten kinetics (apparent K_m ~1640 and ~217 mM, apparent V_max ~5.71 and ~46.9 pmol/mg protein/min, respectively). However, at least two CYPs catalyze 3-OH-CBZ formation and multiple CYPs contribute to 2-OH-CBZ formation (summarized in Fig. 1). After adjustment for relative hepatic abundance, CYP3A4 and CYP2B6 are quantitatively most important in the formation of 3-OH_CBZ. Although carbamazepine 2-hydroxylase activity correlated significantly only with CYP2B6 activity (r = 0.86, p<0.025), inhibitor studies in human liver microsomes and the use of baculovirus-expressed enzymes confirmed that at least five CYPs were significant contributors to 2-OH-CBZ formation; CYP2E1 made the greatest contribution to the Clint of carbamazepine 2-hydroxylation (~30%), but CYPs 1A2, 2A6, 2B6, and 3A4 also made significant contributions (~13-18%) [14].
CYP3A4 plays an important role in the secondary metabolism of both primary, ring-hydroxylated metabolites. CYP3A4 catalyzes 2-OH-IS formation from 2-OH-CBZ at a rate of ~0.405 pmol/pmol P450/min, more than 10 times that of the other enzymes capable of forming 2-OH-IS (CYP1A1, CYP2C19 and CYP3A7) while CYP2B6 and CYP3A5 are essentially inactive. 2-OH-IS can spontaneously oxidize to an iminoquinone metabolite (CBZ-IQ); an S-methyl adduct detected in human urine is thought to be derived from CBZ-IQ conjugation with glutathione [15]. In contrast, the metabolism of 3-OH-CBZ to a metabolite tentatively identified as the catechol metabolite, 2,3-di-OH-CBZ, correlates significantly (r > 0.97) with CYP3A4/5 activities in a panel of human liver microsomes (n=14) Catechol formation is markedly inhibited (>80%) by CYP3A inhibitors, and studies with cDNA-expressed enzymes confirmed that CYP3A4 is the principal catalyst although CYP2C19 appears to play a minor role. Preincubation with 3-OH-CBZ (100 μM) results in time- and NADPH-dependent loss of catalytic CYP3A4 activity (>67%) in both microsomes and cDNA-expressed enzyme. Glutathione (5 mM) does not prevent inactivation, and studies to determine if CYP3A4-dependent biotransformation leads to accelerated, proteasomal degradation of the protein are now being planned.

These data suggest that CBZ can be bioactivated to at least two reactive intermediates – one that is capable of forming glutathione conjugates and one that is associated with CYP3A4 inactivation, and CYP3A4 is a key mediator of both reactions. However, CYP3A4 is also responsible for formation of CBZ 10,11-epoxide, the primary, non-toxic metabolite. Therefore, we speculate that interindividual variability in the relative abundance of each of the three CYP3A4-generated metabolites will be a key determinant in individual susceptibility towards an immune-mediated event (3-OH-CBZ formation and CYP3A4 inactivation/degradation), no toxicity (CBZ 10,11-epoxide formation), or potentially other forms of idiosyncratic toxicity (2-OH-CBZ formation). Application of metabolic approaches, such as computational frameworks to characterize drug biotransformation networks, offers the promise of using kinetic parameters for the formation of each of the three metabolite product derived from individual CYP isoforms to assess the propensity of any given complement of human hepatic CYP isoforms (in which the relative abundance of each member of the complement is varied) to maximize formation of each individual metabolite of interest. It is hoped that this type of analysis will help identify candidate drug biotransformation (bioactivation) genes for future pharmacogenetic analyses.

Bioinformatics and the Response to Xenobiotics

The complexity of idiosyncratic drug reactions implies that multiple susceptibility genes are likely to be involved, with the relative contribution of allelic variation in each of the genes varying among individuals within the “susceptible” population. It is well understood that the expression of many drug metabolizing enzymes and transporter genes are dynamically regulated in response to diet and xenobiotic exposure (medications, environmental chemicals and toxicants) via nuclear receptors such as the Ah receptor, constitutive androstane receptor (CAR) and pregnane X receptor (PXR). It is not unreasonable to expect
that mechanisms to protect the organism following xenobiotic exposure extend beyond drug absorption and metabolism and thus, a comprehensive investigation of pharmacogenetic determinants of susceptibility to idiosyncratic drug toxicity must include allelic variation in all genes involved in the adaptive response to xenobiotic challenge. We have developed new bioinformatics tools based on information theory to model DNA-protein interactions such as those between transcription factors and their cognate response elements in the regulatory regions of modulated genes. Preliminary data generated from a scan of the human genome sequence using a model of PXR/RXR binding (developed with an iterative approach of binding site identification and validation (Fig. 2)) indicate that the genome-wide response to PXR ligands, such as CBZ, includes genes coding for transcription factors, protein and DNA repair mechanisms, and programmed cell (apoptosis).

Summary

Idiosyncratic drug toxicity represents a significant problem affecting patients and their physicians, pharmaceutical companies and federal regulatory agencies in all countries and yet little is understood concerning the factors that place susceptible individuals at high risk for developing this form of drug toxicity. Genomic technologies have fueled the need for new computational approaches to mine and interpret vast volumes of data. The hope for the future is that these new tools will aid in efforts to find creative solutions to complex problems like drug idiosyncrasy and ultimately lead to safer, more efficacious drug therapy.

Acknowledgements

Work in the author’s laboratory is supported by NIH grants from the National Institutes of Health GM58883-03 and ES10855-03.

References

5. Shear NH and Spielberg SP. J Clin Invest 1988;82:1826-1832