High-throughput human metabolism and toxicity analysis

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Poor drug candidate safety profiles are often identified late in the drug development process, manifesting themselves in the preclinical and clinical phases and significantly contributing to the high cost and low yield of drug discovery. As a result, new tools are needed to accelerate the assessment of drug candidate toxicity and human metabolism earlier in the drug development process, from primary drug candidate screening to lead optimization. Although high-throughput screens exist for much of the discovery phase of drug development, translating such screening techniques into platforms that can accurately mimic the human *in vivo* response and predict the impact of drug candidates on human toxicology has proven difficult. Nevertheless, some success has been achieved in recent years, which may ultimately yield widespread acceptance in the pharmaceutical industry.

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Introduction

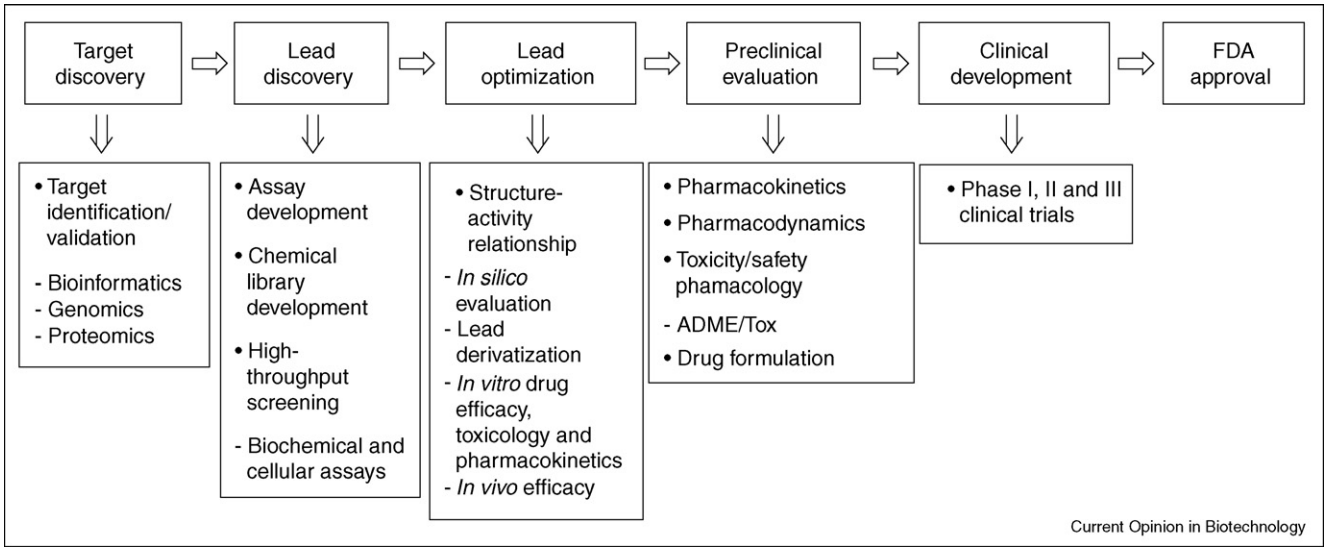
Modern drug discovery is a multidisciplinary enterprise consisting of disease-based target identification and validation in conjunction with high-throughput screening (HTS) of chemical and natural product libraries. This is followed by the careful optimization of selected lead compounds, *in vitro* and *in vivo* pharmacokinetics, toxicology and bioavailability testing leading, finally, to preclinical and clinical studies (Figure 1). In the past decade, there has been a dramatic increase in the number of new chemical entities (NCEs) and screenable drug targets as a result of combinatorial chemistry [1] and advances in bioinformatics [2], genomics [3] and proteomics [4]. Nevertheless, nearly 40% of preclinical and clinical drug candidates fail owing to unanticipated toxicity [5]. Screening for toxicity at early stages of the drug discovery process, if effective, would presumably weed out such failures and enable medicinal chemists to focus their lead optimization programs on drug candidates with acceptable levels of both bioactivity and toxicity. Unfortunately, there has been a lack of *in vitro* technologies that have the requisite throughput to address early-stage toxicology and that can adequately mimic human metabolism to predict the likelihood of drug candidate toxicity [6]. The present discussion addresses state-of-the-art technologies related to high-throughput approaches that can be adopted in early-stage human metabolism and toxicity analysis.

The role of drug-metabolizing enzymes

The human body, primarily the liver, contains a variety of oxidative and conjugative enzymes that are involved in the metabolism of myriad chemicals that comprise today's pharmaceuticals (Table 1). Most important are the cytochromes P450 (CYP450s), which catalyze the first-pass (Phase I) functionalization reactions including oxidation, reduction and hydrolysis [7,8]. Subsequent conjugation reactions (e.g. glucuronidation, sulfation, acetylation and the addition of amino acids and peptides, including glutathione) are catalyzed by Phase II enzymes and result in the formation of soluble compounds that are more readily excreted [9]. Thus, understanding the role of these enzymes in drug metabolism is an important area of research that impacts human toxicology testing.

By far the most important class of metabolic enzymes is the CYP450s, which play several crucial roles in drug metabolism. First, CYP450s are directly involved in the initial clearance of drugs from the body, which reduces the plasma concentration of a drug at a target site and affects the bioavailability in a manner that is often difficult to predict [7,8]. During this process, drug metabolites are generated, some of which are biologically active in their own right and exert the desired pharmacological effect. Often, however, drug metabolism can lead to undesirable biological consequences [8,10]. A well-known example of a toxic metabolic response is the P450-catalyzed oxidation of the common analgesic acetaminophen to *N*-acetyl-*p*-benzoquinone imine, which is hepatotoxic and a major cause of liver failure [10]. Second, CYP450s are often inhibited by drugs or other xenobiotics, which can affect the metabolism of other drugs leading to accumulation and potential toxicity [11]. Finally, inter-individual/populational variability in levels of CYP450s, and even in the induction of CYP450s by drugs, results in tremendous diversity in drug metabolism, producing large variations in a drug's efficacy across a broad spectrum of the population [12].

Figure 1



The conventional drug discovery processes. ADME, absorption, distribution, metabolism and elimination; FDA, Food and Drug Administration.

Phase II biotransformation (with the exception of acetylation and methylation) mostly leads not only to an inactivation of drugs and their reactive metabolites, but also to increased hydrophilicity and thus enhanced excretion. For example, glutathione *S*-transferase (GST) catalyzes the conjugation of reduced glutathione to various electrophilic drug metabolites generated by CYP450 reactions, resulting in detoxification of the reactive metabolites [9]. Thus, inhibition of GST activity and depletion of glutathione levels potentially leads to the deleterious effects of many drug candidates. Another example of a phase II enzyme is UDP-glycosyltransferase (UGT), which catalyzes the conjugation of D-glucuronic acid to various drugs and their metabolites possessing carboxylic acid groups [9]. Indeed, this reaction represents the major route for the elimination and detoxification of drugs. However, it has also been reported that the glucuronidation of certain drugs could lead to more toxic conjugates, which can cause severe hepatotoxicity. Well-known examples of drugs causing a toxic glucuronidation response include nonsteroidal anti-inflammatory drugs (NSAID) such as benoxaprofen and bromfenac [10].

High-throughput biotransformation analysis to assess drug metabolism

The pharmaceutical industry has adopted the 96- and 384-well microtiter plate with liquid-dispensing and plate-handling robotics as the standard workhorse for HTS. The vast majority of HTS techniques are focused on bioactivity testing of a series of compounds using biochemical or cellular screens (Table 2). HTS offers several advantages over larger-scale techniques. These include the need for only nominal amounts of biological target and drug candidate for assay and automation that facilitates parallel sample processing, thus enabling multicomponent screens and multiplexed detection modes [13]. HTS is not common, however, in toxicity testing (Figure 2).

Because of the central role of biotransformation in Phase I and II human metabolism, the high-throughput assessment of biocatalysis is likely to play a significant role in the development of new technologies that can address early-stage toxicology [14,15]. One such example is the rapid identification of CYP450 inhibition, which provides a window on the impact of specific drugs and their known metabolites on potential toxicity and clinically relevant drug–drug interactions [16,17]. The majority of CYP450 inhibition studies are now performed in multiwell plate formats. For example, Trubetskoy *et al.* [18•] employed fluorogenic substrates, which are available for all key human CYP450s, to screen drugs, drug candidates and other test molecules for the potential inhibition of recombinant CYP450 isoforms in high-throughput. CYP450 inhibition assays were performed within an 8 μL reaction volume comprising a specific CYP450, the fluorescent substrate, $NADP^+$ and an NADPH regeneration system, and the test compound. The apparent IC_{50} values (ie. the inhibitory concentration of a compound at which 50% of enzyme activity is decreased) of 19 compounds tested in the miniaturized 1536-well reactions were similar to IC_{50} values obtained for the same drugs in the 96-well plate format, thereby allowing simple cross-format data transition. In addition, the inhibitory activity of the test compounds obtained using fluorescent assays was comparable to that obtained from conventional assays. However, results with fluorogenic substrates have not always correlated well with native CYP450 substrates (e.g. specific