THE BIOLOGY OF CHEMICAL SPACE

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Introduction

The sequencing of the human genome was presented to the public as a watershed moment in science that would have a significant impact on human health. The challenge now is leveraging our knowledge of the linear DNA sequence to understand the complex world of human biology. Small-molecule drug discovery companies have been at the forefront in using molecular methods to tease out relationships between specific biomolecular targets and their roles in disease processes. Those efforts have evolved over the last decade to the point where in vitro bioassays are routinely used to prioritize new chemical entities prior to animal testing. The intersection between selection and optimization of chemical leads and in vitro biology is helping pharmaceutical scientists better understand the biology of chemical space.

A common misconception is that the biological effect of a particular drug is mediated by interaction with a single molecular target. The reality is that virtually all drugs have complex interactions with a large number of biomolecules within the human body. In aggregate, these interactions determine the efficacy and adverse effects of a particular compound. However, testing all potential drug leads on human subjects to reveal these effects is not possible. Historically, animal models were the only surrogate systems available prior to human clinical trials. However, animal testing is relatively expensive, not feasible for testing large numbers of compounds, and raises ethical concerns. In vitro tools provide a useful alternative for understanding the biological properties of potential pharmaceutical compounds.

Developing In Vitro Assays for HTS

The use of in vitro assays for large-scale screening of chemical libraries expanded significantly in the 1990s. Initial work focused on high-throughput primary screening of pharmaceutical companies' proprietary compound libraries. The number of leads generated after initial screens had to be pared down before animal testing. This led to the development of secondary screening assays designed to select compounds with the most drug-like characteristics.

A number of highly publicized adverse drug incidents have also influenced the use of in vitro assays. In particular, the failure of the antihistamines terfenadine and astemizole due to inhibition of the hERG channel has driven pharmaceutical companies to assay for inhibition of this ion channel earlier in the drug-screening process. Other drugs such as cimetidine (H_2 blocker) and mibefradil (calcium channel blocker) have struggled due to their interaction with cytochrome P450 enzymes, leading to nearly universal screening of the major members of this drug-metabolizing enzyme family.

Initial in vitro assays were designed to understand the ADME (Adsorption, Distribution, Metabolism, Elimination) properties of compounds, including their impact on cytochrome P450 enzymes (1). More recently, assays for understanding the in vitro toxicity profile of lead compounds have been integrated into most secondary screening programs (2). In addition to assays designed to test a chemical's effect on a generic target, in vitro methods are also used to understand the selectivity of a compound within a particular target class (3). For example, kinase profiling has found significant utility for understanding the potential off-target effects for this important group of therapeutics. Importantly, recent results suggest that the therapeutic value of some kinase inhibitors is due to inhibition of multiple kinases (4). These recent results highlight the importance of screening a large number of targets early in the drug discovery process.

Chemical Structure and Biological Targets

Efforts are now underway to quantify the relationship between certain chemical classes and their effect on in vitro biology. Most large pharmaceutical companies now have databases on how particular chemical structures affect different biological targets. This information is driving rational drug design during lead optimization. Often, the challenge is selecting the best assay to include in the secondary screening program.

Two major factors that affect the decision-making process are assay development costs and the correlation of assay results with the in vivo biology of the compound. While the utility of any individual assay is difficult to quantify, the consensus of most drug discovery biologists is that the pattern seen when comparing large numbers of related assays run on a number of compounds from a particular chemical series shows significant correlation across the series. By comparing in vitro data with in vivo data generated during clinical studies for compounds in a given series, pharmaceutical companies can select structures that are more likely to be successful in patients. Table 1 shows a profiling screen using two steroids and two calcium channel blockers, which were compared in ten different bioassays. In general, similar compound classes show comparable profiles. The real value of these kinds of data comes from comparing a large number of structurally related compound groups using a suite of related assays, such as multiple cytotoxicity assays. The result is a "bar code" for a particular compound and compound class. These data are similar to those seen with

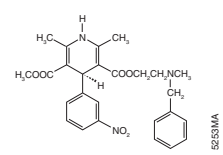
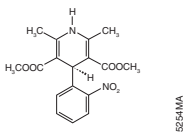
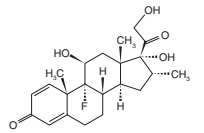
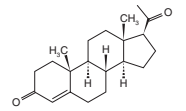
	 Nicardipine	 Nifedipine	 Dexamethasone	 Progesterone
Kinase-Glo® Plus Assay Src Kinase				
PKA				
P450-Glo™ Assay CYP2C19*				
CYP1A2				
CYP2C9				
CYP3A4				
CYP2D6*				
Pgp-Glo™ Assay				
CellTiter-Glo® Assay				
Caspase-Glo® 3/7 Assay				

Table 1. Inhibition (Green) correlates with chemical structure. Red = noninhibitory or stimulatory. Kinase-Glo® Plus Assay was run using the Poly E4Y substrate (Sigma Cat.# P0275) for Src kinase or the Kemptide substrate (Cat.# V5601) for PKA. In the Src kinase assay the IC_{50} was greater than 100µM for nicardipine and nifedipine; the IC_{50} for dexamethasone was 2.67µM and for progesterone was 6.48µM. For the PKA assay, the IC_{50} values for nicardipine, nifedipine and dexamethasone were 6.14, 0.18, and 0.29µM, respectively. The IC_{50} for progesterone was greater than 100µM. CYP450 assays were performed using the P450-Glo™ Assay according to the protocols outlined in Technical Manual #TB340. For the CYP2C19 assay, the IC_{50} values of nicardipine, nifedipine and progesterone were 0.65, 1.3, and 1.6µM, respectively. Dexamethasone had no effect on CYP2C19 under the conditions assayed. For CYP2D6, nicardipine had an IC_{50} of 1.8µM, while nifedipine and progesterone had IC_{50} values greater than 100µM. Dexamethasone did not appear to affect CYP2D6 activity. For CYP1A2, the IC_{50} values of nicardipine and nifedipine were 6.3 and 0.02µM, respectively. Dexamethasone had an IC_{50} of 38.66µM, and progesterone had an IC_{50} of 17.77µM. We used a double-stable HEK293 cell line for the CellTiter-Glo® and Caspase-Glo® 3/7 Assays. Five thousand cells/well were plated, and the drugs were incubated for 4 hours before the assays were run. *P450-Glo™ Assays for CYP2C19 and CYP2D6 are currently in development.

nucleic acid microarrays where any given data point has less value than the aggregate of overlapping oligo hybridizations. The overall profile often provides useful information for predicting the in vivo behavior of a compound.

One of the frustrations for academic pharmacologists has been the lack of accessiblity to the proprietary in vitro assay databases held by drug discovery companies. This issue will likely be alleviated by the development of an annotated public database (PubChem™) containing in vitro assay results generated by the Molecular Library Screening Center Network (MLSCN; see the interview with Jim Inglese, Deputy Director, of the NIH Chemical Genomics Center (NCGC), one of the ten centers of the MLSCN, on page 22). Publication of the results from multiple assays using the MLSCN compound library should help develop biological profiles useful for understanding complex biological questions and validation of new drug targets. This public NIH Roadmap Initiative will help to fulfill the promise of the the human genome project. ■

References

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