

Strategic Importance of Research Support through Pathology

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ABSTRACT

The pace at which new drug candidates are being identified by Discovery Research demands that they be screened for preclinical attributes rapidly and efficiently. The early identification and elimination of compounds with toxic liabilities will produce safer drugs in a shorter time period, and with an increased rate of success. Most major pharmaceutical companies now recognize the strategic role of pathology support for research and have developed specific units to effect this outcome. The early interaction of these pathologists with drug discovery teams to identify compounds with toxic liabilities is critical. Approaches being used include high throughput in vitro screens to predict the relative toxicity of discovery compounds and to provide early indications of underlying mechanisms, target profiling to predict consequences of receptor-ligand interactions at other-than-indicated target sites, and acute in vivo studies to establish tolerability limits and target organs of toxicity. These approaches include the application of contemporary tools such as genomics, proteomics, metabonomics, and genetically engineered animal models. To maximize the benefit of discovery pathology, it is critical that pharmaceutical companies also actively participate in non-proprietary knowledge sharing and the education of pathologists and toxicologists to lead these efforts in the future.

Keywords. Benchmarking; education; investigative pathology and toxicology; in vitro toxicity screens; target organs of toxicity; target profiling; toxicology.

THE PROBLEM

New drug candidates are being identified by Discovery Research at an increasingly rapid pace. Meanwhile, the cost of developing new drugs continues to rise, in large part because the majority of these compounds fail during preclinical testing or clinical trials. Approximately 30% of compounds fail due to toxicity, resulting in increased costs and delays in successful drug development. Recent surveys indicate that it requires 12–15 years and \$500–600 million to bring a single new drug to market. Only 1 in 5–10,000 compounds screened ever successfully reach market and, of those, only 30% produce revenue sufficient to recover the cost of research and development (1, 3).

HOW DISCOVERY PATHOLOGY CAN CHANGE THE PARADIGM

To overcome these problems, the paradigm used to evaluate preclinical attributes of new drug candidates must change. Results of a recent informal survey conducted by the Investigative Toxicologic Pathology Interest Group (see below) indicate that many companies are utilizing discovery pathology to bring about this change. Of nine pharmaceutical companies that responded to the survey (29 companies solicited, 12 respondents, including two biotechnology companies and one contract research organization), eight had specialized in-house units (e.g., discovery, investigative, experimental, or exploratory pathology or toxicology). Pathologists and toxicologists in these units had diverse activities, but were most frequently involved in early screening of compounds (mainly using in vivo models) and in basic research. Conventional morphologic techniques (e.g., light and electron microscopy, immunohistochemistry) were the most frequently used tools,

followed by molecular methodologies (e.g., in situ hybridization) and mechanistic endpoints (e.g., cell proliferation, morphometry).

Establishing early interaction between pathologists and Discovery teams is one of the most critical steps in changing the paradigm. This ensures that discovery pathologists are aware of the team's needs, and conversely, that the teams are aware of the expertise and capabilities available in discovery pathology. Interactions with Discovery teams include a diverse array of activities and, depending upon organizational structures and individual capabilities, it may be advisable to have separate pathologists providing early discovery support as compared to later stages of development, when more traditional toxicological pathology support is needed. Investigative pathology support is also frequently required for mechanistic studies of unanticipated toxicities occurring during later stages of development; however, the current discussion will focus on pathology support prior to compounds entering development. The following is an example of a sequence of interactions between pathology and Discovery that can improve the delivery of compounds with optimal safety profiles to Preclinical Development.

One of the earliest interactions where pathology can support Discovery is target profiling (i.e., defining the distribution of a therapeutic target in various tissues of preclinical species and humans). This information can be used to anticipate possible consequences of altering the pharmacological activity of the target. This process begins with literature and database searches for published results of receptor distribution and receptor-ligand interactions, followed by the use of immunohistochemistry, in situ hybridization, and RT-PCR to identify the distribution of the target in normal tissues. The occurrence of the target in organs other than those intended for therapeutic affect provides early indications of potential target organs of toxicity related to the pharmacology of the drug candidate. Similarly, the early use of genetically engineered mice (transgenics and knockouts) to study gain or loss

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of the function provides valuable information to validate the target or predict toxic sequelae (10). Pathologists play an important role in interpreting the consequences of target profiling results, as well as gross and microscopic tissue changes in genetically altered mice.

A second critical early activity is developing predictive systems for identification of compounds with toxic liabilities before traditional *in vivo* safety assessment studies begin. As part of an initiative termed "Preclinical Profiling in Drug Discovery," Pharmacia has implemented an *in vitro* "Tox Cluster Assay" (7). This system utilizes a rat hepatoma cell line (H4IIE) to assay the affect of compounds on several biochemical processes essential for general cell health. Processes that are assayed include cell proliferation (CyQuant [12]), membrane integrity (α -GST leakage [4]), and mitochondrial function (MTT and Alamar Blue dye reduction [5], and total

ATP [2]). Because these assays are performed at a relatively early stage in the discovery funnel, when compound availability is limited, assays are performed in a 96-well plate format to minimize consumption of compound. All five assays can be completed, including a dose response analysis (0.05–300 μ M) with <5 mg of compound.

An example of a typical application of the Tox Cluster Assay is shown in Figure 1. Tox Cluster results are used by Discovery teams to assist in lead selection and to establish structure-toxicity relationships. At this early stage, Tox Cluster data are used to prioritize, but not to kill, compounds based on a TC_{50} (concentration that produces half-maximal response compared to controls). The data can be further used to predict a sustained plasma concentration (C_{tox} ; no observed effect level at 24 hrs *in vitro*) that would result in toxicity *in vivo*.

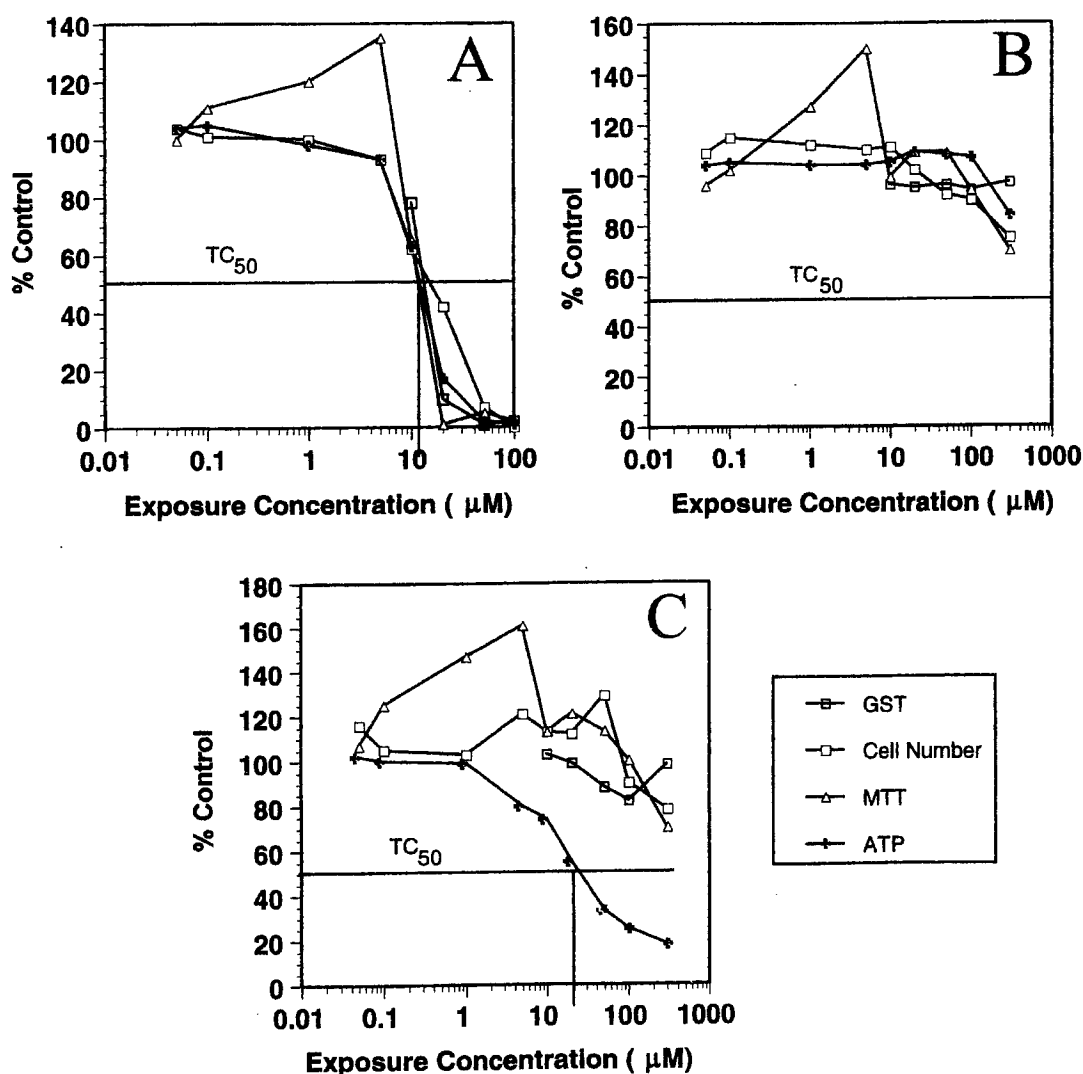


FIGURE 1.—Example of the application of the Tox Cluster Assay to prioritize lead compounds from an anti-infective discovery program (see text for details of the Tox Cluster Assay). The compound in panel A was the most toxic (low TC_{50}); all four parameters tested were affected at a similar concentration and reached maximal response over a narrow range of concentration. The compound in panel B was least toxic (no measurable TC_{50}); minimal effect was seen in all parameters over a wide concentration. The compound in panel C showed no effect on membrane integrity, cell proliferation, or MTT; however, there was a marked concentration dependent reduction in ATP, suggesting a potentially unique mechanism of toxicity (e.g., synthesis or utilization of ATP).

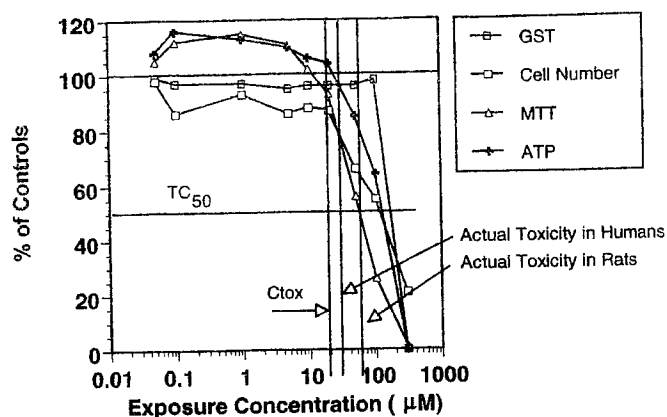


FIGURE 2.—Correlation between toxicity predicted by the Tox Cluster Assay in vitro and hepatotoxicity observed in vivo. Ketoconazole, an antifungal drug with known hepatotoxic side effects, was tested in the Tox Cluster Assay (see text for details of the Tox Cluster Assay). The highest concentration that produced no effect in vitro (C_{tox} , $\sim 20 \mu\text{M}$) was used to predict a sustained plasma concentration that would produce toxicity in vivo. Comparison with published reports showed close correlation with naturally occurring cases of ketoconazole hepatotoxicity in humans ($C_{max} \sim 30 \mu\text{M}$) and in 14-day studies in rats ($C_{max} \sim 60 \mu\text{M}$).

Important issues to be addressed as the Tox Cluster Assay continues to be implemented include; (1) applicability of H4IIE cells for prediction of non-hepatocyte toxicity, (2) comparison of results obtained with transformed H4IIE cells and primary rodent hepatocytes, (3) cross-species extrapolation, (4) ability to detect metabolite-mediated toxicity, and (5) in vivo/in vitro correlations. Initial comparisons between in vitro predictions based on Tox Cluster TC_{50} and C_{tox} values show a close correlation with hepatotoxicity in vivo (Figure 2). Pathologists will play a critical role in correlating in vitro predictions with in vivo observations, and providing guidance for the development of new assays.

An advantage of the Tox Cluster over other in vitro screens is that interpretations are based on results of a combination of assays over a five-log range of drug concentration, rather than any single endpoint evaluated at one or two concentrations. Further, interpreting combined results often provides early clues to the underlying mechanisms of toxicity. Importantly, however, it should be recognized that other approaches and technologies are being used to achieve similar objectives. Amongst these, genomics (9), proteomics (11), and metabonomics (8); (as discussed elsewhere in this series) are important emerging technologies. Different pharmaceutical companies are evaluating or have incorporated these technologies into efforts to establish early predictors of toxicity or efficacy, and to identify biomarkers for use in clinical studies. Some are being pursued through consortia. Two examples are the ILSI/HESI-sponsored Application of Genomics and Proteomics to Mechanism-Based Risk Assessment and Imperial College's Consortium on Metabonomics and Toxicology (COMET).

Having identified potential target organs of toxicity in target profiling studies and prioritized lead compounds using in vitro screens such as the Tox Cluster Assay, pathologists and toxicologists can then more efficiently design early in vivo studies with relatively small numbers of compounds pre-

lected to minimize toxic liabilities. A typical scenario would be to begin with a range-finding study (e.g., single dose, 3 dose groups representing multiples of efficacious dose, 3 rats/group, 1-day duration) to determine general limits of acute tolerability and to collect toxicokinetic data. Clinical observations, gross necropsy findings, and exposure data are then used to design a sub-acute study (e.g., repeated dose, 2 dose groups representing maximal tolerated dose and a fraction thereof, 6 rats/group, 7-day duration) to more clearly define target organs of toxicity. These studies are conducted relatively early in the discovery funnel when compound is still limited. For example, the two studies described can typically be completed with <10 grams of drug.

This sequence of events is designed to optimize the safety profile of compounds entering traditional preclinical development and regulatory-driven toxicologic pathology studies. By doing so, cycle time is reduced because less unanticipated toxicity occurs during development and the success rate is increased because compounds with greater potential toxic liabilities do not enter development.

FUTURE DIRECTIONS

The value of research support through pathology to optimize the safety profile of Discovery compounds is becoming increasingly important in the pharmaceutical and biotechnology industries. Recent advances have come from broad-based in vitro screens to assess general biological processes. Additional technologies need to be evaluated and developed to screen for specific organ toxicities. For example, there is a current need for screens to predict cardiotoxicity resulting from compounds that prolong the QT interval (6). Similarly, strategies to assess the risk of immunotoxicity are currently being closely evaluated by regulatory authorities.

The greatest gains will be made if companies willingly benchmark their activities and share non-proprietary knowledge bases. The Investigative Toxicologic Pathology Interest Group (ITPIG) is a good example. ITPIG consists of a group of pathologists and toxicologists from several dozen pharmaceutical and biotechnology companies that have been meeting informally since 1998. The purpose of ITPIG is to benchmark strategies and practices used for investigational toxicology and pathology studies, with the aim of achieving industry-wide success through appropriate sharing of intellectual resources and collaboration in establishing research standards. ITPIG has emerged as a useful forum for discussion of contemporary topics and dissemination of information. A survey to assess pathology and toxicology efforts in Discovery (see above) is an example of one of the group's activities. In addition, ITPIG has contributed to the development of several recent ILSI Seminars and portions of ACVP Annual Education Meetings.

Finally, given the changing role of pathologists and toxicologists in the drug discovery/development process, it is important that companies sponsor and actively participate in the education of new scientists to lead these efforts in the future. Several companies have made individual programmatic contributions to such efforts. Unfortunately, there is no central organizing body to coordinate advertising, funding, and recruiting activities. Consequentially, educational institutions and students usually become aware of these programs

only through personal contacts. Closer involvement of industry and institutional training programs represents a unique opportunity to educate a pool of highly qualified pathologists and toxicologists specifically trained to provide research support.

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