Data gaps limit the translational potential of preclinical research

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The absence of mouse pharmacokinetic reference data hinders translation. An analysis of recent literature highlights a systematic lack of discussion regarding rationale for the selection of dosing paradigms in preclinical studies, and in particular for neuroscience studies in which the lack of brain penetration can limit target-organ exposure. We propose solutions to improve study design.

Despite widespread use of pharmacological agents in mouse models of human disease, the literature lacks comprehensive pharmacokinetic profiles for such studies. Coupled with a paucity of suitable data are shortcomings in the training of experimental biologists in the application of pharmacometric principles to experimental study design. Many authors simply cite previously published studies to support the selection of a particular dose, even when the cited paper lacks drug exposure data. There is an assumption on the part of researchers that if a referenced study demonstrates a biological effect—that is, any measurable physiological or behavioral effect—in a rodent at a given dose, then that same dose will also effectively perturb disease-relevant mechanistic biology in a different study. The danger occurs when the observed therapeutic effects are not linked to drug-induced mechanistic alterations at the level of the target organ. Lack of a drug exposure—response relationship in a target organ casts doubt on mechanistic interpretations. In addition, any changes in the route of drug administration, vehicle preparation, species used (rat versus mouse versus primate), age or strain of animal, transgenic modification, time points under investigation, duration of dosing, or organ targeted for intervention (for example, brain versus a peripheral tumor) can alter the relation between dose, exposure, and measured response. In such cases, assumptions regarding the mechanistic basis for observed therapeutic effects may not hold true.

Preclinical pharmacological experiments that do not measure drug concentrations in the target organ run the risk of producing exposures that are too low or too high to interpret a mechanistic hypothesis. Most drugs are not selective, over a large exposure range, for a single molecular target. Confident evaluation of a therapeutic hypothesis requires an understanding of the drug’s penetration and kinetics within the target tissue, as well as its potency and selectivity for specific molecular targets. Further, investigators must consider the concentration of the unbound fraction of drug that is available to interact with the target. Published reports often overlook the fact that many small molecules are more than 90% bound to plasma or tissue proteins, which greatly decreases the fraction of drug available to bind to the intended target. Thus, in cases in which drug binding has a slow off-rate, an organism’s total drug exposure is not a predictor of drug available to interact with its target (1). The failure of some academic scientists to obtain relevant pharmacokinetic data impairs the interpretation of preclinical research results and likely contributes to the acknowledged difficulties in replicating some academic literature, as reported by industry scientists (2, 3).

Drug discovery teams in industry settings routinely collect pharmacokinetic data to aid in the mechanistic interpretation of in vivo preclinical data and to project optimal dosing paradigms for efficacy and toxicity studies. Data required to evaluate brain penetration are not typically collected by industry-based drug-discovery teams for compounds originally developed for therapeutic indications that do not obviously implicate the central nervous system, making this information especially hard to find for many otherwise well-described drugs. In addition, because mouse data are not required for preclinical toxicity studies (the more common small animal species for preclinical toxicity being rats), industry scientists do not often obtain pharmacokinetic data from mouse experiments. These issues are especially relevant for older drugs that are potentially suitable for repurposing. Many older drugs were discovered and characterized before routine pharmacokinetic-pharmacodynamic (PK-PD) modeling of preclinical drug exposure and its application to predicting human dosing became standard practice. Last, pharmacokinetic data are not considered innovative, and these studies generally do not achieve publication in peer-reviewed journals, even when the data have been generated. When such data are published, it is often relegated to the unsearchable black hole of supplementary materials. Thus, mouse neuroPK profiles are not readily available for many drugs that are frequently used in conjunction with mouse models of human brain disorders.

DOCUMENTING DOSING STRATEGIES

To evaluate the potential impact of insufficient pharmacokinetic data on dose selection in a sample of recent published neuroscience literature, we conducted an analysis of papers identified by means of a PubMed search using the search terms “drug” and “brain” for the publication year 2014 from eight journals (Table 1). This list was culled to include only primary research reports that included systemic administration of a pharmacological agent, a pharmacological therapeutic, or a biological therapeutic as part of the study design. The search yielded 100 articles published between 1 January and 30 December 2014 that used systemic drug delivery with the intended goal of targeting the brain of rodents (table S1). Each publication was examined for the stated rationale behind the dose selection of study drugs (Table 1).

The reported rationale for dosing strategies fell into several broad categories, including (from lowest confidence to highest) (i) dose selected, rationale not discussed; (ii) literature citations of another study in which reports ranged from citation of exposure in the same species, exposure in a different strain or species, a dose conversion from the human literature to rodent, or reports of effects on rodent behavior in another study; (iii) demonstration of an effect on rodent behavior or function in the current study; (iv) demonstration of a dose-responsive biological effect in the current study; (v) measurement of drug levels in blood or plasma in the current study; and (vi) measurement of drug levels in the target organ (that is, the brain) in the current study. In only two instances were publications identified that considered the impact of drug binding...
to plasma or brain proteins on the free exposure of drug available to interact with the target. This is a critical flaw in most published studies that use small molecules for functional effects in the brain because many central nervous system (CNS) drugs that penetrate the blood-brain barrier exhibit high protein binding, leaving a small fraction of the total drug measured in plasma or brain unbound and free to interact with the molecular target. Furthermore, most studies used evidence of a biological activity to justify dose selection without consideration for how exposure of the agent relates to the potency of the compound at known molecular targets, which would be required to test a mechanistic hypothesis.

The lack of pharmacokinetic consideration does not imply that every study used an inappropriate dose of drug to test their hypothesis. It does illustrate that a clear rationale was not provided for dose selection in most publications. Furthermore, all 11 of the 100 publications that measured total brain exposure included an author from the pharmaceutical industry (n = 5), an academic drug screening group (n = 3), or a pharmacology–pharmaceutical sciences department (n = 3). This observation likely reflects the limited presence of pharmacology and pharmaceutometrics departments within most academic institutions and limited access to the mass spectrometry and other analytical resources needed to measure drug levels in study samples. Outsourcing the bioanalysis of samples collected from study animals is feasible, but the use of contract research organizations to support such studies is often too costly for most academic grant budgets to accommodate.

**DATABASES AND REPURPOSING**

Recent years have seen increasing efforts to investigate approved or clinically tested drugs for new indications (4–8). Such repurposing has been touted as a means to accelerate therapeutic development (4). For example, a strategic pillar of the U.S. National Institutes of Health’s (NIH’s) translational roadmap calls for the academic community to actively participate in the repurposing of drugs approved by the U.S. Food and Drug Administration (FDA) or investigational drugs that have passed safety hurdles but failed in clinical trials because of lack of efficacy (9–11). To have a meaningful impact in neurological and psychiatric disorders, such drug repurposing efforts will require access to neuropharmacokinetic (neuroPK) data sets in mice to guide the testing of new therapeutic hypotheses in genetically engineered disease models. A recently published consensus evaluation of drug repositioning opportunities for Alzheimer’s disease identified 15 potential drug candidates. These were further prioritized for testing on the basis of available evidence to produce a shortlist of seven compounds reviewed by industry experts to provide insight into the viability of these candidates. The most common shortcoming identified for the compounds considered was issues related to insufficient brain penetration or the lack of information about optimal dosing strategies (11).

The repurposing of statins illustrates how the neuroPK knowledge gap limits progress. Statins were developed as 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors to lower cholesterol and reduce...
risk of cardiovascular disease (12). FDA has approved at least nine different statins, and most are commonly prescribed; nearly one-third of Americans ages 55 to 64 took a prescription cholesterol-lowering drug between 2009 and 2012 (www.cdc.gov/nchs/data/hus/hus14.pdf). The widespread availability and safety profile of statins has lured researchers into evaluating their potential for repurposing (13). Statins have been profiled extensively in preclinical research to test for potential therapeutic benefit in Alzheimer’s disease (14–19), Fragile X syndrome (20), Rett syndrome (21, 22), epilepsy (23), Huntington’s disease (24), Parkinson’s disease (25, 26), stroke (27), and brain injury (28, 29).

A search of the literature reveals no systematic neuroPK studies in any mouse strain that would enable direct comparisons of CNS exposure across the various statins. In silico predictions based on the drugs’ molecular properties suggest that the nine most widely prescribed statins each have a different potential to penetrate the blood-brain barrier, different potencies against the HMG-CoA reductase enzyme, and different “off-target” activity profiles (30). On the basis of available data, there is reason to believe that simvastatin has the best overall profile for inhibiting HMG-CoA reductase in the brain (30). A recent study reported that lovastatin is able to reverse a range of phenotypes in a mouse model of Fragile X syndrome (20). However, the design of an optimal clinical trial will require the collection of mouse pharmacokinetic data to understand how much CNS drug exposure is required to produce efficacy in the disease model. There are at least two possible scenarios. Given that simvastatin is more potent at inhibiting HMG-CoA reductase than are other statins and likely to be more brain penetrant in both mice and humans, one would expect that simvastatin will be more potent than lovastatin in ameliorating symptoms in both mice and humans if the observed efficacy stems from inhibition of HMG-CoA reductase activity in the brain by lovastatin. The advantage of this outcome would be that better brain penetration and potency would lead to a lower overall dose requirement to achieve efficacy, and thus likely a better safety profile.

A second scenario could be that lovastatin is more potent than simvastatin in the mouse model of Fragile X syndrome because of an additional biological activity inherent to the lovastatin molecule, which may not yet be documented in the literature. In either case, understanding the CNS exposure of lovastatin required to produce efficacy in the mouse will determine whether there is a safe therapeutic index for achieving the required concentration in patients. Previous attempts to discern useful neuroPK parameters from the literature for the use of statins in rodent models have highlighted the lack of critical data as the looming roadblock to progress in the field (31, 32). Until these data exist, the translational potential of preclinical research may be limited. And this is but one example of one drug class.

The creation of a centralized database is needed for the entire translational research community and would establish a new mechanism for academia, funding agencies, foundations, and industry to pool resources. If studies are done well the first time and documented in an open-access resource, it will reduce redundant efforts and improve the quality of decision making by scientists considering innovative solutions to our biggest health problems.

FILL THE GAPS

Manuscript submission practices for several high-impact journals now include requirements that authors include detailed information regarding study design and statistical analysis with each submission. A reasonable extension of this checklist should include the stated rationale for doses selected for study drugs. Information should include a discussion of data highlighted in Table 2. Authors should be expected to reference a relevant data set from a high-quality database or publication, or provide the data in the current study (Table 3).

Industry biologists learn basic principles of medicinal chemistry, pharmacokinetics, and drug disposition while working on drug discovery project teams. Academic groups are playing an increasing role in translational therapeutics and, in particular, drug repurposing. Academic programs need to augment training in pharmacokinetics and pharmacodynamics so as to increase the rigor of preclinical work and to ensure that investigator-initiated clinical studies are testing hypotheses effectively. Institutions without a department of pharmacology or pharmacometrics might lack the organizational knowledge needed to conduct drug studies and must identify resources or collaborators to patch these deficits. Formal coursework and Web-based resources and tutorials are needed to train and support translational researchers.

Support translational researchers. Manuscript and grant reviewers need to demand higher standards for preclinical studies with respect to reporting on drug exposure associated with biological effects. Ethics committees responsible for review of animal protocols should require investigators to provide rationale for dose selections in proposed studies. Similarly, scientific review boards at academic medical centers need to include clinical pharmacologists who are able to review investigator-initiated clinical studies to ensure that proposed dosing strategies will test a meaningful hypothesis.

Table 2. Recommendations for use of pharmacokinetic data. The first column includes a list of recommended data sets to aid reviewers of submitted articles in the interpretation of preclinical findings. The second column includes a list of useful reference data that would support improved preclinical study design in mice if available in a public database.

<table>
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<tr>
<th>Literature reports that evaluate study drugs should include:</th>
<th>Compound-specific data that should be included in a rodent pharmacokinetic database:</th>
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<tbody>
<tr>
<td>• Expected or measured plasma exposure of the study drug in the preclinical species during the study</td>
<td>• Elimination half-life (T_{1/2})</td>
</tr>
<tr>
<td>• Expected or measured target organ exposure of the study drugs in the preclinical species during the study</td>
<td>• Systemic clearance (CL)</td>
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<tr>
<td>• Expected or measured free fraction (unbound by protein) of the study drugs in the target organ of the preclinical species during the study</td>
<td>• Fraction of drug that is protein bound (f_{p})</td>
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<td>• Expected or measured potency of the study drug against the hypothesized activity in vitro</td>
<td>• Maximum plasma concentration after drug administration (C_{max}) and time to reach maximum plasma concentration (T_{max}) for a standardized dose and route of administration</td>
</tr>
<tr>
<td>• Expected or measured potency of molecule at known biological targets</td>
<td>• The ratio of drug in brain to that in plasma (B/P)</td>
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<td>• The ratio of drug found free in brain (C_{fu,b}) to that found free in the plasma (C_{fu,p}) defined as C_{fu,b}/C_{fu,p}</td>
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<td></td>
<td>• Any potential impact of drug transporters (found on the rodent blood-brain barrier) in limiting brain exposure</td>
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A central repository that contains brain penetration, protein binding, and pharmacokinetic profiles of drugs and pharmaceutical tools in rodents is needed to effectively support translational research. This database should also provide basic tutorials that define primary pharmacokinetic parameters with examples to illustrate how data are used to predict optimal dosing strategies. The minimum data set needed for each compound in a useful rodent database is highlighted in Table 2. Access to this information and supporting materials will have an immediate impact on the quality of translational drug repurposing efforts across brain disorders, and will support the development of new therapeutic approaches to neurological disorders and mental illness. Existing databases managed by NIH or precompetitive consortia could be reinforced with donated pharmacokinetic data sets and tutorials.

Industry and government scientists should work precompetitively to collect and curate pharmacokinetic data sets in conjunction with supporting educational materials. Mouse pharmacokinetic data exist inside pharmaceutical companies for a wide range of publically disclosed molecules and literature standards. Release of these data into a public database would provide several benefits to companies including (i) increased scientific rigor in the literature with a higher probability of reproducibility; (ii) increased appreciation by the academic biology community for the difficulty inherent in generating molecules with potency and pharmacokinetic profiles suitable for in vivo work, opening the door for in-kind collaboration with academic groups; and (iii) direct comparison of data collected in-house to that collected at other companies or institutions to enable better internal quality control. Comprehensive pharmacokinetic data sets will benefit all therapeutic areas, regardless of whether the brain is the target organ, because peripheral and central exposure data can be generated from the same experiments. Moreover, the principles described above for the CNS apply to other target tissues in which vascular barriers, metabolic processes, or active transport alter the distribution of systemically administered drugs.

Key to ensuring that preclinical mouse studies test the hypotheses they aim to evaluate is an understanding of the unbound fraction of drug present in the target organ at an appropriate time point under study. Grant and journal reviewers need to carefully consider whether authors of proposals and manuscripts are providing adequate rationale for their choices of preclinical dosing paradigms. Importantly, the collection and centralization of rodent pharmacokinetic datasets will promote efficient generation of future data, reduce the collection of redundant data, and improve the return on investment for research funds that are devoted to preclinical studies aimed toward clinical translation.

**Supplementary Materials**

Table S1. One hundred publications that used systemic drug delivery with the goal of targeting rodent brains.

**Table 3. Recommendations to improve translation through the use of preclinical pharmacokinetic data.**

<table>
<thead>
<tr>
<th>Journals:</th>
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<tr>
<td>requirement authors to provide explicit rationale for dosing strategies used</td>
<td>Rationale should include consideration of the unbound drug exposure in target organ as best practices</td>
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<th>Education:</th>
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<tr>
<td>include basic pharmacology and pharmacokinetic principles in formal coursework required for basic preclinical and clinical research scientists</td>
<td>Develop tutorials and on-line calculators for rodent dose projections to support appropriate use of published pharmacological tools</td>
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<th>Databases:</th>
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<td>reinforce public chemical databases with mouse pharmacokinetic data that includes brain exposure</td>
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<th>Precompetitive consortia:</th>
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<tr>
<td>create precompetitive consortia to solicit mouse pharmacokinetic data sets from industry and foundation partners for database expansion</td>
<td>Targeted data collection for compounds already in the public domain</td>
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**References and Notes**


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