Drosophila for drug discovery: useful tool or wishful thinking?

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Introduction. In the quest for new effective drugs there is the push for faster ways to identify active lead compounds to develop and validate in drug discovery pipelines. Cell culture-based screens are easy to run and can be both standardized and automated to perform efficient high-throughput screens. Combined with the large available compound libraries, they can be useful to identify leads. However, many of such leads have been found to fail in the subsequent validation steps, where system complexity increases, with many compounds revealing as toxic to a multicellular organism or unable to penetrate the target organs.

Flies are increasingly and successfully being used for pharmacological studies (Freires et al., 2016) as demonstrated by the highly attended Fly Pharmacology workshop at the 2018 *Drosophila* Research Conference in Philadelphia earlier this year. In this crowded venue, it emerged that there appears to be a substantial bias against using flies for drug discovery, which is manifested in frequent proposal rejections and caustic comments from reviewers. Here I will forego extensive discussion of the worth of *Drosophila* studies, as this community is well-aware of the strength of this model. However, I want to reflect on some of the common concerns and share our experience in hopes to encourage the use of flies in drug discovery and ideally to start a discussion or reflections on this topic that may help to strengthen grant proposals and build strategies for the success of drug discovery research in *Drosophila*.

Whole-animal screens in drug discovery and development. Whole-animal screens can efficiently reveal toxicity and can thus eliminate many false positives at an early stage, saving time and expenses, remaining aware that, depending on animal model, screening and protocol administration used some active compounds may be missed because of improper absorption and permeability issues. Concerns have been raised regarding the real significance of the results in model organisms and how they may translate to humans. This is particularly true of the invertebrate disease models *Coenorhabditis elegans* and *Drosophila melanogaster* that have, however, demonstrated remarkable successes in pharmacological assays. Key to these achievements were the careful use of the disease model and proper definition of the experimental question, which are paramount in comparative and model organism studies. In fact, mammalian models, particularly mice where a large statistic is available, have yielded results that do not always translate to humans. Thus, warnings about overreliance on mice models have been voiced (Strange, 2016). Vertebrate models demand high maintenance costs ranging from about one thousand dollars per year for one mouse and 2500 dollars for a rat, to approximately 40,000 dollars for one monkey, and are subjected to ethical concerns. Moreover, both disease mechanism and drug's mechanism of action are often largely unknown and relatively intractable in mammalian models, reducing experimental power to little more than careful description of the processes. While important, descriptive knowledge scarcely contributes to mechanistic knowledge of the disease and drug action. In search for productive ways forward, *Drosophila* appears to offer key advantages as drug discovery model. For example, *Drosophila* has excellent genetic conservation, the equivalent of most human organs (unlike *C. elegans*) and has sophisticated genetic tools that can be used for mechanistic studies. Both disease mechanism and drug mechanism of action can be probed in the fly. In comparison, zebrafish, a popular vertebrate model for pharmacology, has much less

developed genetics and is aquatic, which may impact certain studies (*e.g.*, kidney pharmacology). *Drosophila* short life span and culture economy lends itself well to longevity studies and to monitor the consequences of prolonged drug administration, potentially decreasing costs of the drug discovery pipeline that may become prohibitive in other animals. While long-term drug response monitoring in complex vertebrates could in principle be implemented by investing with bold funding programs, current high-throughput pharmacological screens often remain brute-force approaches with low success rates. Therefore, in the long term it seems prudent to work towards improving efficiency of the drug pipeline, ideally by integrating data from interdisciplinary research on multiple systems to accelerate discovery. The resulting improved success rates would free up resources to tackle more diseases. Because these efforts have been embraced by the scientific community, there is the need for dialog and sharing thoughts and experiences to fully comprehend this new field with its obvious strengths and yet-to-be-defined limitations.

Common approaches and concerns for fly-centered drug discovery. Drug development requires structure-activity relationship studies, which involves experimental iterations to determine which positions in the molecule can be modified to optimize drug properties. For expedience, analytical amounts of synthetic compounds should ideally be used in this phase, which can be conveniently be achieved in whole-animal models using flies.

Chronic disease appears to pose a particularly complex problem for pharmacology. Compared to forms of aggressive cancer, for example, chronic disease may take longer to become life-threatening and may require maintenance regimes with long-term drug administration. In these conditions, even slight toxicity may become a serious concern which reduce effective drug options, highlighting the need for long-term toxicological studies.

When using flies in drug discovery, one important concern regards possible laborious drug administration. The insect cuticle may present a barrier to administration of certain drugs and microinjection has been used successfully in the quest for drugs targeting the nervous system. While conveniently done, microinjection is laborious and may limit high- and medium-throughput drug-screening efforts. Other forms of administration such as spraying are also possible and can be automated (Pandey and Nichols, 2011). Oral administration, on the other hand, can be easily and qualitatively monitored by mixing the drug with food and coloring agents, the ingestion of which can be seen through the semi-transparent cuticle of both adults and larvae. If deemed important, a fluorescent compound can be added instead and used as a proxy to quantify ingested amounts. It is often feared that flies will reject certain drugs, but that may not be a frequent occurrence. In our experience, we have administered 24 different compounds of four different chemical families including peptides, peptide derivatives and different small-molecule drugs, used both alone and in combination. We never found an instance of drug rejection as determined with food-coloring-spiked mixes of food and drugs. Speaking with other colleagues, we can report anecdotally that drug ingestion seems to be a common occurrence, suggesting that drug rejection may be infrequent and possibly limited to some pungently smelling molecules.

It is likely that oral administration may require higher compound dosage compared to microinjection, due to in-animal drug processing and may even result in either inactivation of certain drugs (*e.g.*, unmodified peptides) or even drug uptake by the yeast in which the drug is often mixed prior to being fed to the flies. While remedial use of yeast extract instead of whole cells may be

attempted in the latter case, the ease of oral administration and the small scale of fly-based drug assays make the use of possibly higher doses of compounds much more attractive than microinjection. We found that dose-response of rapamycin administration to a fly model of polycystic kidney disease indicated an effective dose of 12.5 μM, which is nine to ten times higher than those injected into mice models (Gamberi et al., 2017). Albeit this particular proof-of-principle experiment only represents a single instance, we noticed that this concentration of orally-administered drug was within one order of magnitude of the doses injected into mice, which may optimistically be considered as suggestive of possible similar range of activity in the two systems, at least for rapamycin. Future investigations will likely provide more information to evaluate this possibility.

In our experience it has always been useful to perform dose-response assays in the fly to ensure drug activity. Precise drug dosage on the other hand, is regarded as largely species-specific due to the exact aspects of physiology typical of each species. Thus, model organisms, including *Drosophila*, may occasionally guide drug dosage range, but are not considered valid guidelines *a priori*. In exciting new developments, flies have shown conservation of certain drug-binding sites (Ziehm et al., 2017) and of toxicological pathways (Zhou et al., 2017) corroborating the accumulating evidence for *Drosophila* being a *bona fide* model for drug discovery in which toxicological studies are also possible. Signs of activation of conserved toxicological pathways can be monitored over time via -omics approaches that can also simultaneously enable the basic study of how cellular pathways respond and adapt to drug treatment, doubling the return on investment of these types of studies.

Conclusions. We are in the pioneering days for fly pharmacology in which healthy skepticism abounds, yet the accumulating evidence suggests that flies can be useful models in which to accelerate drug discovery, identify good-quality lead compounds and ultimately provide indications of drug efficacy for specific conditions. The research frontline recognizes the need to invest into validating the use of invertebrate models in drug discovery. Studies of global responses to chemical treatments in different genetic backgrounds will help to precisely define the boundaries of what is possible to achieve using fly models combined with clever choices of experimental questions based on basic comparative knowledge. Considering the current trend of successes, fly pharmacology promises to be here to stay.

References

Freires I. A., De Cassia Orlandi Sardi J. And R. Dias De Castro 2016, Pharm Res 34(4):681-686. doi: 10.1007/s11095-016-2069-z

C. Gamberi (corr.), Hipfner D. R., Trudel M., Lubell W. D., 2017, PLoS Genet. 13(4):e1006694. doi: 10.1371/journal.pgen.1006694.

Pandey U.B., and C. D. Nichols 2011, *Pharmacol Rev* 63:411–436.

K. Strange 2016, ILAR Journal, 2016, 57(2):133–143

Zhou S., Luoma S. E., St Armour G. E., Thakkar E., Mackay T. F.C., and R. R. H. Anholt 2017, PLoS Genet. 13(7):e1006907. doi: 10.1371/journal.pgen.1006907.

Ziehm, M., Kaur S., Ivanov D. K., Ballester P.J., Marcus D., Partridge L., and J. M. Thornton, 2017. Aging Cell, doi:10.1111/acel.12626.