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STATE-OF-THE-ART REVIEW

Using Zebrafish for High-Throughput Screening of Novel Cardiovascular Drugs

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SUMMARY

Cardiovascular diseases remain a major challenge for modern drug discovery. The diseases are chronic, complex, and the result of sophisticated interactions between genetics and environment involving multiple cell types and a host of systemic factors. The clinical events are often abrupt, and the diseases may be asymptomatic until a highly morbid event. Target selection is often based on limited information, and though highly specific agents are often identified in screening, their final efficacy is often compromised by unanticipated systemic responses, a narrow therapeutic index, or substantial toxicities. Our understanding of complexity of cardiovascular disease has grown dramatically over the past 2 decades, and the range of potential disease mechanisms now includes pathways previously thought only tangentially involved in cardiac or vascular disease. Despite these insights, the majority of active cardiovascular agents derive from a remarkably small number of classes of agents and target a very limited number of pathways. These agents have often been used initially for particular indications and then discovered serendipitously to have efficacy in other cardiac disorders or in a manner unrelated to their original mechanism of action. In this review, the rationale for in vivo screening is described, and the utility of the zebrafish for this approach and for complementary work in functional genomics is discussed. Current limitations of the model in this setting and the need for careful validation in new disease areas are also described. An overview is provided of the complex mechanisms underlying most clinical cardiovascular diseases, and insight is offered into the limits of single downstream pathways as drug targets. The zebrafish is introduced as a model organism, in particular for cardiovascular biology. Potential approaches to overcoming the hurdles to drug discovery in the face of complex biology are discussed, including in vivo screening of zebrafish genetic disease models. (J Am Coll Cardiol Basic Trans Science 2017;2:1–12)

The identification of novel drugs for cardiovascular disease is a major challenge. Many of today's cardiovascular drugs are designed to modulate well-known "legacy" targets, in essence pathways far downstream, such as blood pressure, membrane stability, and lipid levels, which may have limited specificity for the underlying disease mechanism. Many such drugs are only modestly effective or are limited in their utility by "on-target" and "off-target" toxicity (1–5). For example, the focus of the current antiarrhythmic armamentarium is the modulation of myocardial automaticity, refractoriness, or conduction. Importantly, these are also the fundamental components of myocardial biology required for the maintenance of a regular rhythm. The clinical strategies applied in therapy for arrhythmias ranging from simple atrial premature beats to malignant ventricular tachycardia are remarkably similar, as a consequence of the lack of definitive mechanistic insight into many clinical arrhythmias.

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The resultant targeting of "final common pathways" or even normal physiology with blunt pharmacological tools rather than the precise manipulation of disease-specific mechanisms leads to predictable problems (6–8). Not surprisingly, many effective antiarrhythmic agents are also highly proarrhythmic in particular contexts, and these "on-target" adverse effects have become all too apparent, with several costly failures in large randomized clinical trials (3,9,10). In this paper, we outline evidence that most existing cardiovascular drug targets are poorly validated, review emerging data on the role of mechanistic insight in drug discovery for specific cardiovascular diseases in humans, and discuss recent advances using direct in vivo chemical screens in zebrafish for the discovery of novel cardiovascular tool compounds and drug leads.

CHOOSING CARDIOVASCULAR TARGETS

Chronic cardiovascular diseases pose several fundamental problems for drug development (1,11). Clinical events may present abruptly and often with severe consequences (arterial occlusion, paroxysmal arrhythmia, venous thrombosis, or complex vasomotor syncopal events), but the underlying myocardial, vascular, or systemic substrate may be totally undetectable by conventional technologies (12–16). Indeed, the investigation of many chronic cardiovascular disorders is characterized by difficulty in making a negative diagnosis. This dilemma has driven cardiovascular medicine to exploit the concept of risk factors, treating higher risk cohorts identified by specific downstream biomarkers but without overt manifestations of disease, to enable the prevention of disorders (17).

Arrhythmias are an excellent paradigm for much of common complex cardiovascular disease because they are often paroxysmal, limiting the utility of direct approaches to detection and confounding rigorous evaluation of pharmacological or other therapeutic interventions. For many clinically significant arrhythmic syndromes, lifetime risk that an episode will occur may be quite low, but the risk from each individual paroxysm for a morbid or mortal outcome may be quite high (18). Similarly, for arterial occlusive events, even the presence of existing partially obstructive lesions is not a particularly effective predictor of subsequent acute events (19). In addition, the lack of accessibility of cardiac and vascular tissue has left the field to focus on cross-sectional assessment of the final anatomy or physiology, while upstream causal molecular or cellular biology is largely uninterrogated. The balance of risk and benefit in the face of long-term exposure to agents with limited efficacy, even with only a small possibility of severe toxicity, is biased against net benefit. Many clinical trials include aggregates of multiple constituent disorders into single syndromes and so also have likely diluted the effects of new medications, impeding the progress of new drugs that in very heterogeneous conditions must meet very high thresholds of proof. Heterogeneity of etiology results directly in heterogeneity of individual effect sizes, with consequent implications for the magnitude of clinical trials and their costs. These same constraints have also limited enthusiasm for discovery programs for cardiovascular drugs in the pharmaceutical industry (1,20). However, the clinical significance of cardiovascular diseases, and their associated mortality and morbidity, continues to dominate other disorders, including even cancer.

At the core of these concepts is an implied need to carefully balance the risks of a specific condition and the risks of any new therapeutics throughout the development process. These concepts have been framed under the rubric of precision or individualized medicine, which recently has become the focus of major federal initiatives (21,22). Real individualization of medicine dictates remarkable changes in almost everything that we do. It will require a new wave of studies to define the etiologic basis of each disease subset, mechanism-specific diagnostics, transformative approaches to disease modeling, and drug discovery on a previously unimagined scale (23). Although this is well under way for clonal neoplasia, it will not be feasible in the management of chronic cardiovascular diseases until we have robust approaches to the identification of fundamental mechanisms; detection of subclinical disease; cost-effective, efficient, and predictive disease models; and truly scalable approaches to drug discovery in mechanistically faithful models (22–24).

BEYOND TRADITIONAL TARGETS

To date, cardiovascular discovery has focused on a limited repertoire of molecular targets. In myocardial disease, almost every successful agent has transferred from the antihypertensive field, even in situations in which there are intrinsic cellular myocardial abnormalities such as hypertrophic cardiomyopathy (20,25,26). In arrhythmias, almost all of the activities in drug discovery have been focused on transmembrane ion fluxes and the associated channels or ion exchangers required to generate these (27). In vascular disease, the focus has been on modulating lipids and directly influencing the mechanisms of