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Opinion

Cellular Biomechanics in Drug Screening and Evaluation: Mechanopharmacology

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The study of mechanobiology is now widespread. The impact of cell and tissue mechanics on cellular responses is well appreciated. However, knowledge of the impact of cell and tissue mechanics on pharmacological responsiveness, and its application to drug screening and mechanistic investigations, have been very limited in scope. We emphasize the need for a heightened awareness of the important bidirectional influence of drugs and biomechanics in all living systems. We propose that the term 'mechanopharmacology' be applied to approaches that employ *in vitro* systems, biomechanically appropriate to the relevant (patho)physiology, to identify new drugs and drug targets. This article describes the models and techniques that are being developed to transform drug screening and evaluation, ranging from a 2D environment to the dynamic 3D environment of the target expressed in the disease of interest.

Trends

An argument is outlined for a new interdiscipline: mechanopharmacology.

Examples of cellular biomechanics influences on drug action are described.

The relevance of matrix stiffness and of internal and external stresses to drug screening is discussed.

Mtethods for the biomechanical perturbation and analysis of single cells and organoids are reviewed.

Drug Screening and Evaluation: The Need To Consider Cellular Mechanics

The reasons for failure of drug development programs are the subject of much contemplation. Although adverse effects, toxicity, as well as pharmacokinetic features, are often cited as reasons for arrested drug development, several recent studies highlight failure because of lack of efficacy. A review of the portfolio performance of AstraZeneca in Phase IIa and IIb studies from 2005 to 2010 suggested that 57% and 88%, respectively, of the project closures at this stage were due to a failure of efficacy, whereas attrition due to lack of efficacy in the preclinical phase was as low as 6% [1]. There are many reasons to expect that preclinical and clinical pharmacology will differ, including the use of non-human species to support efficacy. However, even when the target is expressed and engaged in human cell types, failure may ensue because the affected pathways are less influential than anticipated from the preclinical studies. When the agent reaches the target in adequate concentration and for a sufficient duration, giving a suitable level of drug exposure, lack of efficacy is likely to result from differences in behavior of the drug target in the assay systems compared with the target behavior *in situ* in the patient-specific context. The screening and preclinical pharmacology for many of these agents is likely to have been established in cell culture, in an oversimplified mechanical microenvironment, and/or in non-human models of the targeted disease. We argue that drug screening can be improved with the use of human cells of phenotype most relevant to the condition, ideally being derived from patients (representative of the disease stage being targeted), and then cultured in the most (patho)physiologically relevant conditions. This approach is intended to ensure that the assay emulates the biomechanical environment in the condition to be treated. Ideally, the assay would also embed cell mechanical measurements of deformability, stiffness, and/or contraction, as in many organs and diseases, because these cellular changes often constitute the principal endpoint of therapeutic intent. The use of patient-derived primary cell cultures improves the

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likelihood of genetic and epigenetic influences being appropriately expressed, with the expectation that the target efficacy would be more accurately predicted. It will be valuable to rigorously test this proposition against comparator preclinical efficacy testing in relevant animal models and in 2D culture on a rigid plastic substrate.

The impact of biomechanics on cell function has been systematically explored, leading to a broad appreciation of mechanosensitive processes, with the principal mechanosensors being selected ion channels [2,3] and less commonly the integrins [4]. Mechanotransduction involves force transmission through bound proteins resulting in conformational changes that entrain functional impacts. For example, conformational changes in vinculin and talin have been shown to subserve the recruitment of the actin cytoskeleton to focal adhesions in the leading edge of migrating cells [5,6].

The impact of biomechanics on drug actions is rarely addressed, despite being highlighted as an important consideration repeatedly in the literature (e.g., [7,8]). Recent advances proposed by Donald Ingber and colleagues using 'organ-on-a-chip' microfluidics technology involving cell cultures being subjected to cyclical strains (breathing/cardiac cycle/peritoneal peristalsis/renal fluidic shear) raise the prospect of more systematic and relevant drug discovery paradigms using human cell cultures [9,10]. Similarly, recent advances in cell mechanics have highlighted the suitability of mechanical endpoints as phenotypic targets in high-throughput screening [11]. In this article we develop selected examples of biomechanical impacts on cell function and drug responsiveness, and discuss refined, biomechanically appropriate bioassays, emphasizing those suitable for scaling to medium to high throughput.

We exemplify below the selected impacts of different aspects of the biomechanical environment (Box 1)

Shear Forces

The effects of shear are extensively explored in the cardiovascular system [7], but there are other organs where fluid and gas flows create shear forces that impact on cell and tissue function. Shear represents the frictional force exerted by flow of gas or liquid over the affected surface and is quantitated in terms of force (Dynes) per unit area (Box 1).

One of the most instructive exemplars of the interaction between drug action and shear stress is provided by the discovery of excess cardiovascular mortality associated with the use of cyclooxygenase 2 (COX2)-selective inhibitors (coxibs). Two COX enzymes are known. COX1 is ubiquitously expressed at significant levels and produces precursors for the formation of prostaglandin E_2, prostacyclin (PGI_2), and thromboxane A_2 to achieve cytoprotective, anti-atherogenic, and hemostatic physiological functions, respectively. COX2 was discovered in tumor cells and has been shown to be strongly induced by particular cytokines, growth factors, and receptors for pathogen-associated molecular patterns (PAMPs) in the mammalian innate host defense system.

The basis of the anticipated safety profile of selective COX2 inhibitors was in part dependent on a misapprehension of the dependence of vascular endothelial PGI_2 production on COX1 activity. The anti-thrombotic actions of PGI_2 were well-established a decade before the 1990 discovery of COX2. The mechanisms of anti-thrombotic actions of low-dose aspirin were known to involve preserved endothelial PGI_2 production and diminished production by platelets of the platelet-activating vasoconstrictor, thromboxane A_2. In 1996, 2 years before the coxibs were approved by the USA FDA, work by Gimbrone and colleagues indicated that, under static conditions and with the application of turbulent flow, cultured endothelial cells expressed COX1, whereas when subject to laminar flow, COX2 expression was strongly induced and therefore became an