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Development of an in vitro platform using the human primary cardiomyocyte work loop assay to screen for drug-induced effects on cardiac contractility

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Drug-induced changes in cardiac function is a common cause of compound attrition. The level of contractility of the cardiac muscle is also referred to as its inotropic state, while the rate of contraction can be referred to as its chronotropic state. Furthermore, the ability of a cardiac muscle to relax following peak contraction is referred to as its lusitropic state. The level of cardiac contractile state directly contributes to cardiac output and therefore the maintenance of blood perfusion throughout the body. A decrease in cardiac contractility reduces perfusion to vital organs, while drug-induced increases may lead to increased workload and oxygen demand, both of which can lead to increased mortality. The significance of adverse drug effects on cardiac contractility is often dependent on the safety margin. Available in vitro assays are poorly predictive of the concentrations that may affect contractility in humans and furthermore, current in vivo contractility measurements are often invasive, resource/time consuming, low throughput and indirect. Therefore, there is a need for more predictive in vitro assays that have a higher throughput than available assays. We have demonstrated that the Work Loop cardiac contractility assay is more predictive of human findings than existing assays. We have also recently demonstrated that the Work Loop cardiac contractility assay is highly predictive of inotropy risk to man. Recently we have expanded this investigation to determine whether the cardiomyocyte Work Loop assay had the potential to provide a predictive, higher throughput model of heart muscle dynamics to assess inotropic effects. Normal cardiac force generation is the product of several interrelated and interdependent physiological processes at the cellular and organ levels. Propagating action potentials originating at the sinoatrial node have a generation rate that is modulated by the autonomic nervous system. These action potentials at the cellular level activate transmembrane ionic currents that include influx of calcium ions. The resultant elevation of intracellular-free calcium (calcium transient) induces the release of internal calcium stores from the sarcoplasmic reticulum that, in turn, leads to the release of inhibitory tropomyosin complexes from interdigitating actin and myosin myofibrils, allowing them to slide along their long axis to elicit cell shortening. Active, ATP-dependent reuptake of intracellular calcium into the sarcoplasmic reticulum by energy-dependent SERCA leads to cell re-lengthening and a state of relaxation. The human heart consists of billions of muscle cells (cardiomyocytes, CMs), that rhythmically contract in order to provide the mechanical force for pumping blood throughout the body. The rhythmic contraction of the heart is regulated by a wave of electrical activation, which is based on the excitability of cardiac cells and their ability to generate and propagate action potentials. An action potential (AP) is a characteristic transient change in the transmembrane voltage that starts with a depolarization phase and ends with a repolarization phase. Pacemaker cells located in the sinoatrial (SA) node regularly depolarize by themselves and generate spontaneous APs, a property that is referred to as automaticity. In contrast, atrial and ventricular CMs are quiescent, have a resting state and can generate an AP only if the transmembrane voltage is raised above a certain threshold value by an external stimulus. Still, the CMs are electrically connected via gap junctions, which are ion conducting channels formed by proteins. Hence, the currents created during an AP of a cell may flow to and stimulate an AP in the neighbouring cells. Once a cell has produced an AP, there is a refractory period during which the cell can generate no further AP. Together, excitability and refractoriness of cardiac tissue ensure that the wavefront of depolarization, initiated by the pacemaker cells, is propagated in a unidirectional manner from the atria through the atrioventricular (AV) node to the ventricles. Development of a human heart primary cardiomyocyte contractility assay could greatly improve the understanding of the human relevance of nonclinical findings. We have developed novel isolation protocols for adult human cardiomyocytes. The myocytes retain rod-shaped striated morphology, contracting in response to field electrical stimulation. We have developed stable cardiomyocyte work loop assay protocols which remain stable and enable cumulative concentration drug responses to be applied. To characterize adult human cardiomyocyte contractility, we are assessing a range of reference inotropic agents and effective concentrations compared with those tested in the clinic. Interestingly, the multiparametric readout allowed for the differentiation of inotropes operating via distinct mechanisms. Hierarchical clustering of contractility transient parameters, coupled with principal component analysis, enabled the classification of subsets of both positive as well as negative inotropes, in a mechanism-related mode. Thus, human cardiomyocyte contractility model could accurately facilitate informed mechanistic-based decision making, risk management and discovery of molecules with the most desirable pharmacological profile for the correction of heart failure. The Work Loop assay has the potential to be a new approach to the detection of inotropic drug effects on cardiac contractility. Assays that provide greater concordance with man are crucial in the assessment of cardiac safety in drug development.

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