

CELL BASED AFM BIOSENSING FOR SCREENING OF PULMONARY-DRUG RELATED ARRHYTHMIC EFFECTS

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Abstract

Atomic force microscopy (AFM) combined with stem cell derived human cardiomyocytes (CM) enables dynamic follow-up of cardiac contractions (e.g. beating rate, contraction and relaxation times), simultaneously with other CM biomechanical properties. Today, majority of drugs entering clinical usage needs to be tested for adverse arrhythmic effects; nevertheless, the effects on cardiomyocyte contraction are not routinely employed, only when related to cardiac pathologies. AFM-based biosensor allows in-vitro disease modeling, but also enables to monitor the effect of CM-contraction affecting drugs. Until today only few selected drugs modulating contractility and spontaneous pacing were described in animal models. This work for the first time demonstrates that basic biomechanical parameters, such as average value of contraction force and the beat rate, represent valuable pharmacological indicators of different phenotypic effects on cells without genetic burden. The presented method is robust and has low computational requirements, while keeping optimal spatial sensitivity (force detection limit 200 pN, corresponding to 20 nm displacement). The cardiac stimulating activities of drugs utilized in pneumology as aminophylline, ipratropium, and salbutamol were tested. Stimulating drugs, e.g. methylxanthines and caffeine, presented aberrant cardiomyocyte response, confirming arrhythmogenic potential, and force related fluctuations. Quantification of spontaneous contraction irregularities and related contractility changes allow precise scaling of potential negative effects adding new safety level to clinically relevant drug testing. AFM combined with human CMs serve as robust real-time screening platform for effects of pulmonary drugs. Here we describe changes in CM contractility, which is hard to describe by other screening methods and was never tested with described medication.

Keywords: Cardiomyocyte, contraction, arrhythmia, in vitro modeling, drug adverse events, pulmonary drugs

1. INTRODUCTION

There is large group of patients with chronic obstructive pulmonary disease (COPD) and bronchial asthma (AB), receiving long-term/lifelong and combined bronchodilator (BD) treatment. Majority of commonly used drugs in this combination has certain influence on heart contractility and rhythm. The most prevalent drugs include methylxanthines, beta-2-sympathomimetics (β 2-SM), parasympatholytics, and inhaled or oral/intravenous corticosteroids [1,2].

Salbutamol, a β 2-SM, provides relief of shortness of breath and other symptoms for relatively short period of about six hours. Nevertheless, a number of cases of abuse was, reported due to its supposed anabolic effects and muscle performance enhancement [3–8]. Nowadays, there is a number of case studies and experimental body of evidence that during long-term BD treatment by β 2-SM, parasympatholytics and methylxanthines, some patients [or the substance abusers] develop cardiomyopathy (CMP), chronic heart failure (CHF), cardiac arrhythmia (CA) and even sudden cardiac death (as the first cardiac manifestation of CMP) [9–17].

Cellular and even cardiomyocyte (CM) interactions with BD drugs were studied and are well-known from animal models and primary CMs mixtures (homogenized animal hearts) [18,19]. Despite wide array of phenotypic assays available [20] human CMs have not yet been studied and it remains to be disclosed, how BD substances affect their physiological functions, especially contractility and rhythmicity.

Unique application of atomic force microscopy (AFM) as nanomechanical transducer combined with stem cell derived human CMs enables dynamic monitoring of cardiac contractions [21–23]. CMs beating rate, contraction and relaxation times and absolute force values can be recorded simultaneously with other relevant biomechanical properties [24]. Human CMs gain role in drug development, improving attrition rates [25], especially cardiotoxicity assays [26–29]. Adult human CMs, are not readily accessible from obvious reasons, still CMs can be differentiated from either human embryonic or induced pluripotent stem cells (hES and iPS, respectively) [30]. In this pilot work, we present an electromechanical in vitro system of 3D cardiac models and AFM together for drug testing in the format of an (organ) heart-on-a-chip. For this reason, we have used clinically relevant concentrations of selected drugs aminophylline (methylxanthine), salbutamol (β 2-SM) and ipratropium (parasympatholytic).

2. METHODS

The hES line CCTL14 derived and characterized at Masaryk University, Brno [31] was used. Cardiac differentiation was performed as previously described [30,32,33] with minor modifications, resulting in 3D spheroids of regular size and shape, called embryonic bodies (EB). AFM setup (JPK NanoWizard 3, JPK, Berlin, Germany), together with an inverted light microscope (IX-81S1F-3, Olympus, Tokyo, Japan), was employed to record mechanocardiograms of spontaneously contracting EBs as previously described [21–23]. The tested drugs were diluted to clinically relevant concentrations [19,34] and the resulting response curves were recorded after initial equilibration in Tyrode's solution, followed by sequenced exchanges of media with increasing drug concentrations.

Each measurement consisted of 10 min stabilization and 10 min measurement. The mechanical properties of beating EBs were expressed as force of contraction in nN (R-S) as previously described [30]; however, the values were relativized for final comparison. Rhythmical properties were described as contractions/beats per minute (BPM). Statistical evaluation was carried out in GraphPad Prism 8.4.3 software (GraphPad Software, Inc., La Jolla, CA, USA). All data were tested for outliers by ROUT (Q = 1 %) method, and available normality tests were performed. Contraction and force of EBs with different concentration of aminophylline was cross-compared by Brown-Forsythe ANOVA test, with Dunnett's T3 multiple comparison test. Due to single measured entity, statistics were not applied to salbutamol and ipratropium.

3. RESULTS

Pilot sets were measured for 12 clusters of CMs derived from hES cell line CCTL14. Total of 10 clusters were measured in four subsequent concentrations of aminophylline, nevertheless 3 of those exhibited aberrant behavior, previously described as stop-and go effect [23]. These were excluded from the pilot evaluation. Only pilot sets were measured for Salbutamol and Ipratropium (so far).

Force (RS) and BPMs were not significantly differing, probably due to small sample size; nevertheless, the observed trend has shown increased force and frequency for high concentrations (10 mM) of Aminophylline.

Moreover, the initially compact set of contractions became highly variable. On the contrary, small Aminophylline concentrations (10 μ M) led to insignificant decrease of contractility and force. Details are depicted in **Figure 1**.

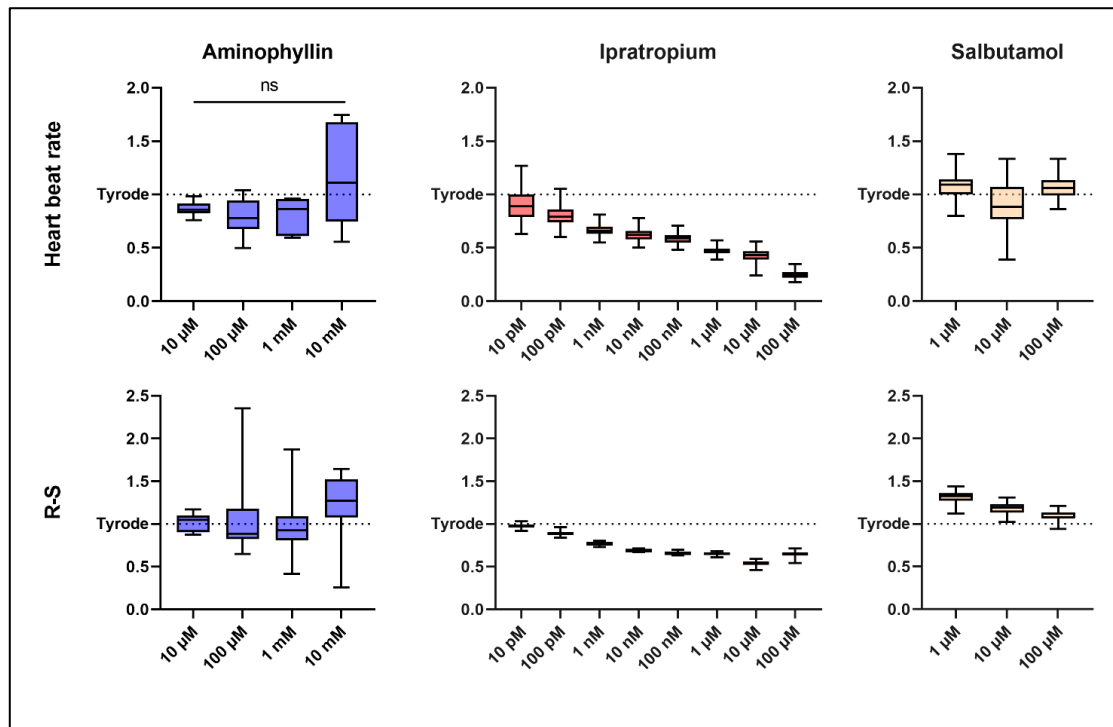


Figure 1 relativized force of contraction in nN (R-S) contractions/beats per minute (BPM)

Salbutamol in pilot trial did not exhibit change in contraction rate, nevertheless small concentration of Salbutamol resulted in 1.5 fold increase in contractility, which negatively correlated with increasing concentration of the substance up to 100 μ M.

Ipratropium showed sustained negative trend through all concentrations, in both contraction rate and force.

4. DISCUSSION

This pilot set of data has for the first time shown consistent trend in CMs behavior influenced by pulmonary drugs. The Aminophylline end-point function is a phosphodiesterase inhibitor; thus an increase of intracellular calcium levels should be present, contributing to positive inotropy [35]. Nevertheless, those changes can be also ascribed to direct catecholamine effect [36]. This limited set of data would support opinion, that Aminophylline does not significantly contribute to myocardial contractility [37]; however, it may have some role in increasing contraction rate and the effect on the regularity of contraction has to be further studied.

Salbutamol on the other hand has shown negative trend for inotropic response, which is in contrast with positive inotropic effect in animals (guinea pig and dog), despite describing lower-than-Isoprenaline effect [38]. Difference may be attributed to lower concentrations used in animal experiments (less than 10 μ M), which may correspond to the first (ascending) part of contractility curve. Here may be shown the same, but descending part of curve, after reaching maximal inotropic effect of the substance. Chronotropic effect of Salbutamol was ascribed to atrioventricular (AV) nodal increased conduction [39,40], rather than sinoatrial node (SA). Increase of the beating frequency in the whole organ can also relate to lower refractory period in the large piece of tissue, enabling easier arrhythmia occurrence and spreading [40]. Nevertheless, its direct effect on SA node is very small compared to AV node and this seems to be also irrelevant to the 3D CM model of small scale,

where the spontaneous contractions are independent of conductive system. Increase in rhythm of contraction cannot be traced in the pilot Salbutamol dataset.

Positive chronotropic effect of ipratropium is attributed to decrease of parasympathetic vagal stimulation, typical for anticholinergic drugs [41,42]. One work described a small decrease of contraction frequency due to other muscarinic agonist (Tiotropium), Although no significant change was traced directly to ipratropium [43] Contrary to this, we have observed sustained decreased chronotropic and inotropic effects of the substance; further investigation has to expand the number of samples and provide more data beyond the pilot experiment.

5. CONCLUSION

This pilot set confirmed that AFM-based nanomechanical system combined with cardiomyocytes can serve as a robust screening platform for direct drug effects, especially myocardial contractility, which is hard to describe by other screening methods. Pulmonary drugs with stimulating properties such as aminophylline, presented aberrant cardiomyocyte response in this context. This is a clear sign of arrhythmogenic potential of distinct compounds. Moreover, force related fluctuations were present in pilot data *in vitro*. Spontaneous contraction irregularities quantification and related contractility changes allow precise scaling of potential negative effects, adding new safety level to subsequent clinically relevant drug testing.

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