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High throughput bioassay for beta1-adrenoceptor autoantibody detection

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ABSTRACT

Background: While the involvement of adrenergic beta1-autoantibodies (beta1-AABs) in pathogenesis of cardiomyopathies is well established as are the benefits associated with autoantibody removal by immunoapheresis, the development of drugs neutralizing beta1-AABs *in-vivo* has been slowed due to a lack of high throughput autoantibody analytics. Highly scalable routine diagnostics involving immobilized binding partners have mostly failed in comparison to the laborious bioassays, which are difficult to scale up, but present the most reliable and sensitive tools for detecting the beta1-autoantibodies.
Methods: A high throughput, image-based assay to measure cardiomyocyte beat rate and contractility was developed and tested for its applicability for detecting adrenergic beta1-autoantibodies. The classical bioassay of spontaneously beating neonatal rat cardiomyocytes was used for comparison.
Results: The high throughout assay using human iPSC-derived cardiomyocytes was able to detect beta1-AAB activity of biological sample material. The results from the high throughput assay were very similar to the data obtained from the original bioassay of spontaneously beating neonatal cardiomyocytes, with one exception, where a control antibody targeting the N-terminal end of the human beta1-receptor induced a response when tested with the high throughput imager, while none was observed by the classical bioassay. This discrepancy may be explained by the differences in host species of cardiomyocytes tested by the two methods.
Conclusion: The high throughput system using iPSC-derived cardiomyocytes for the detection of beta1-AAB provides a realistic option to overcome the sample-size limitations of the bioassay-based diagnostics.

1. Introduction

Autoantibodies against beta1-adrenoceptors are gaining acceptance as essential factors in the pathogenesis of a variety of cardiomyopathies, particularly dilated cardiomyopathy (DCM). "*In the United States, dilated cardiomyopathy occurs in approximately five to eight people per 100,000; it causes approximately 10,000 deaths and 46,000 hospitalizations each year. It is the most common reason for heart transplantation.*" [1]. DCM manifests with a disturbed contraction function of one or both ventricles accompanied with its dilation.

Although, removal of such autoantibodies by various apheresis procedures has demonstrated clear benefits [2,3,4,5,6,7], these strategies have failed to attract a greater clinical interest due to the application complexity, cost, and burden on the patient.

Lack of alternative therapeutic options and a dearth of diagnostic procedures for the beta1-AAB identification [8] have slowed the progress in this field. Thus far, bioassays based on the beta1-AAB mediated beta1-adrenoceptor (beta1-AR) activation are the only reliable techniques to identify the beta1-AAB presence in the serum of affected patients. While a peptide-based ELISA technique was proposed for detecting beta1-AAB, the validity of this method was questioned [9]. One of the first bioassays developed and still widely used, utilizes spontaneously beating neonatal cardiomyocytes and a simple reverse phase contrast microscope for the detection of beta1-adrenoceptor agonists and antagonists, including the agonistic acting beta1-AAB [10]. However, bioassays are laborious, time consuming and inefficient with a low sample throughput and therefore unadaptable for routine diagnostic protocols for large number of samples.

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[1] These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.
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We have developed a high throughput (HT), image based assay to measure cardiomyocyte beat rate and contractility. The HT imager captures a series of digital images of beating cells from 12 wells of a 384-well plate at a time and can analyze an entire 384-well plate in 40 min. Four replicates readings from each of the 12 wells can be obtained in a short period of 20s and is highly efficient compared to manually counting the beat rate with the classical bioassay technique. We tested the feasibility of using a beta1-AAB-detecting bioassay on cardiomyocytes in the high throughout format using beta1-AAB samples from various species and of different purity grades. Data were compared to the ones obtained from the original bioassay of Wallukat and Wollenberger [10]. Our results indicate that the HT imager can accurately detect beta1-AAB samples and has a great potential for use in diagnostic applications.

2. Material and methods

2.1. Antibodies

2.1.1. Rabbit polyclonal beta1-AR-AB containing IgG fraction

Polyclonal rabbit beta1-AABs were produced according to German animal welfare regulations by a commercial antibody developer, BioGenes GmbH Berlin, Germany. The immunogen sequence, EYGSFFCELARR, corresponds to the sequence of the 1st loop of the adrenergic beta1-receptor in rat and human (EYGSFFCEL) and is also the epitope sequence for the beta1-AAB against the 1st extracellular loop in DCM patients (dominant epitope SFFCEL, followed by EYGSFF) [11]. Polyclonal rabbit antibodies specific for the N-terminal end of the adrenergic beta1-receptor were generated using the following sequence: VPASPPASLLPPASESPEPLSQQWC.

IgG containing serum was drawn 1–6 month after immunization for both antibodies. The IgG fraction was prepared as described under IgG preparation.

2.2. Human IgG/beta1-AAB

2.2.1. Sample material

Beta1-AAB containing immunoglobulin-eluate from the regeneration of IgG-immunoabsorption column from a DCM patient was obtained from the Deutsches Herzzentrum, Berlin, Germany. The IgG-eluate (elution with 0.2 M glycine-HCl buffer, pH 2.8) was neutralized immediately using 1 M Tris-buffer pH 7.4. Control IgG from serum of healthy donors was obtained from a blood donation facility and was tested beta1-AAB negative. The patient and the donors signed an informed consent form.

2.2.2. IgG preparation

To one milliliter of the neutralized eluate fraction or the respective serum control material, 0.66 mL of a saturated ammonium sulfate solution was slowly added under agitation. The mixture was incubated for 18 h at 4 °C, followed by centrifugation for 15 min

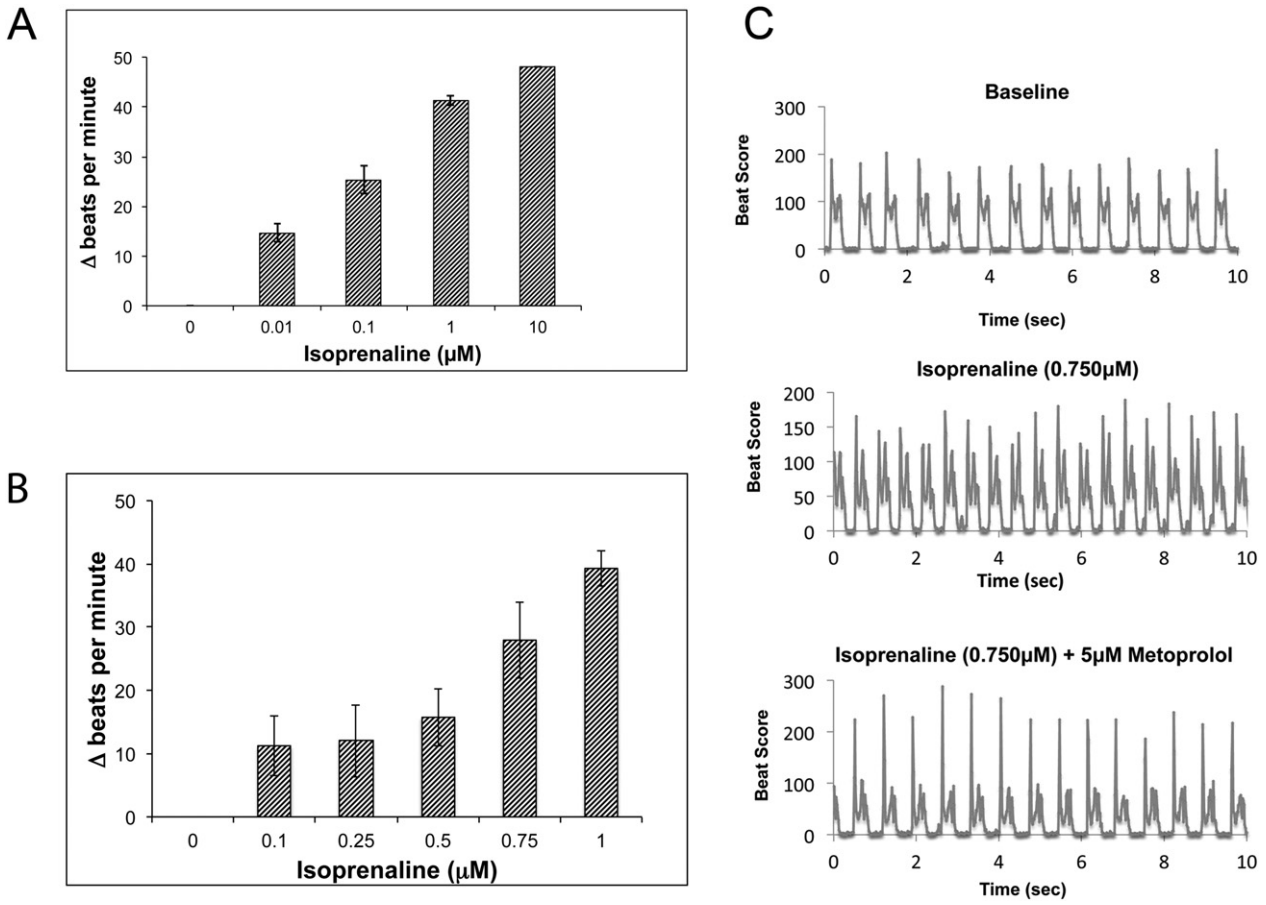

Fig. 1. Chronotropic cell response to the beta1-AR receptor agonist isoprenaline. A) Classical bioassay of spontaneously beating rat cardiomyocytes. B), C) Human Cor4U® cardiomyocytes in the high throughput assay system. Addition of 5 μM metoprolol reversed the chronotropic effect of isoprenaline. The classical bioassay data are the mean of two independent assays, six independent cell clusters each. For the high throughput assay, data from three independent wells for each concentration were averaged.