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(54) Title: IGF-1 (INSULIN-LIKE GROWTH FACTOR) AS THERAPY FOR CARDIAC ARRHYTHMIAS

(57) Abstract: Methods for the treatment of arrhythmia includes the step of administering a therapeutically effective amount of IGF-1 or an analog thereof, or in general any PI3K activating / upregulating agent, to a patient who exhibits one or more symptoms or indicia of cardiac arrhythmia.

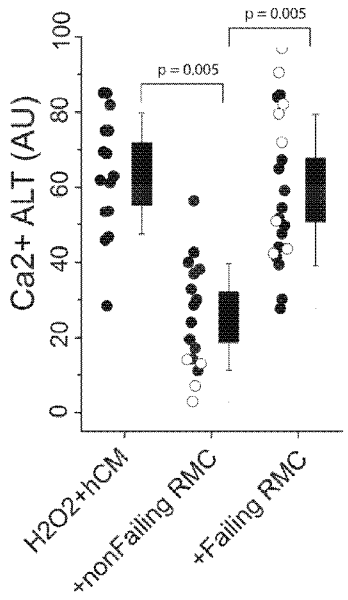

FIG. 6

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IGF-1 (INSULIN-LIKE GROWTH FACTOR) AS THERAPY FOR CARDIAC ARRHYTHMIAS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/572,077, filed October 13, 2018, which is hereby fully incorporated by reference herein.

BACKGROUND

[0002] The present disclosure relates to methods for treating or preventing cardiac arrhythmias and associated conditions in a patient. The methods of the present disclosure comprise administering to a subject in need thereof a therapeutically effective amount of a composition comprising IGF-1.

[0003] The human heart includes muscle walls, valves, vessels, and an electrical system that work together to pump blood through the body. Various conditions can affect cardiovascular function. In particular, a cardiac arrhythmia is a rhythm disturbance, or irregular heartbeat, caused by a malfunction of the heart's electrical or conduction system. This conduction system, shown in **FIG. 1**, transmits impulses from nodes in the heart at a controlled rate. An electrical signal starts at the sino-atrial (SA) node **20** and travels through the atria **22**, causing them to contract. The signal continues down through the atrio-ventricular (AV) node **24**, through the bundle of His **26**, and finally through thin fibers **28** that spread the signal outward to the specialized conduction system **30** of the ventricle muscle. The muscle fibers are connected to cells of the ventricle walls **32**. As the impulse spreads across the cells, the muscles contract to push blood to the body.

[0004] Cardiovascular disorders are the most common cause of death in the United States. Morbidity and mortality associated with heart disease and abnormal heart rhythms (i.e. arrhythmia) remain a public health dilemma in the United States, and in an aging population with increasing prevalence of metabolic disorders, outcomes are likely to worsen. For example, ventricular tachycardia (VT) is a fast (but regular) heart rhythm that starts in the ventricles. Diagnosis of VT is by an electrocardiogram (ECG) showing a rate of greater than 120 beats per minute (bpm) and at least three wide QRS complexes

in a row. VT can be classified as non-sustained versus sustained based on whether or not it lasts less than or more than 30 seconds.

[0005] If left untreated, some forms of ventricular tachycardia may progress into ventricular fibrillation, where the heartbeats are so fast and irregular that the heart stops pumping blood, which can be life-threatening. Diagnosis is usually by an electrocardiogram (ECG) showing irregular unformed QRS complexes without any clear P waves.

[0006] Cardiac alternans, a beat-to-beat oscillation of the T-wave in an electrocardiogram, is closely associated with the occurrence of arrhythmia. Prolongation of action potential duration (APD) is also a known cause of arrhythmia.

[0007] Additional treatments against cardiac arrhythmias would be desirable.

BRIEF DESCRIPTION

[0008] The present disclosure relates to the use of insulin-like growth factor 1 (IGF-1) for the treatment and prevention of cardiac arrhythmias.

[0009] Disclosed herein are methods for the treatment of cardiac arrhythmia of a patient, comprising: administering to the patient a therapeutically effective amount of IGF-1 or an analog thereof.

[0010] The patient can be identified by a finding of cardiac alternans in an electrocardiogram of the patient, or by a finding of ventricular tachycardia in the patient, or by a finding of ventricular fibrillation in the patient, or by other methods that predict high arrhythmia risk (e.g. family genetic history).

[0011] Alternatively, the patient can be identified by finding a level of IGF-1 or a marker of oxidative stress in a biological sample obtained from the patient and comparing the level of the IGF-1 or the marker of oxidative stress to a threshold value. The biological sample may be blood. The marker of oxidative stress may be a reactive oxygen species (ROS), or a redox molecule that counterbalances a ROS; or a protein or a biomolecule.

[0012] The IGF-1 or analog thereof can be administered intramuscularly, intravenously, intracoronary, intraperitoneally, parentally, or orally. In particular embodiments, the IGF-1 or analog thereof is PEGylated.

[0013] Also disclosed are methods for the treatment of cardiac arrhythmia of a patient, comprising: administering to the patient a therapeutically effective amount of a drug that contains the active portion of IGF-1.

[0014] Also disclosed are methods for the treatment of cardiac arrhythmia of a patient, comprising: administering to the patient a therapeutically effective amount of a PI3K activating agent.

[0015] These and other non-limiting features of the present disclosure are discussed in more detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0017] **FIG. 1** is a cross-sectional view of the heart, illustrating how an electrical impulse spreads across the heart to contract the chambers.

[0018] **FIG. 2A** is a set of two electrical traces from different experiments. The top trace is for human cardiac myocytes only (hCM). The bottom trace is for human cardiac myocytes that are co-cultured with human mesenchymal stem cells (hCM+hMSC).

[0019] **FIG. 2B** is a graph showing cardiac alternans in the hCM and hCM+hMSC groups. The presence of hMSCs significantly reduced Ca^{2+} alternans. The y-axis is in arbitrary units, and runs from 0 to 0.8 in increments of 0.1. ($n=9$, $p < 0.0001$).

[0020] **FIG. 3** is a graph showing cardiac alternans in human cardio myocytes (hCM) exposed to H_2O_2 to simulate disease conditions. The leftmost bar is for the hCMs without any drugs (control). The center bar is for hCMs in the presence of IGF. The rightmost bar is for hCMs in the presence of VEGF. The y-axis is in arbitrary units, and runs from 0 to 0.8 in increments of 0.1. * = $p < 0.05$.

[0021] **FIG. 4** is a graph showing ELISA results for IGF-1 levels measured in bone marrow mesenchymal stem cells (BM MSC); normal resident mesenchymal cells (RMCs) obtained from human hearts without heart failure (Normal RMC); and RMCs obtained from human hearts with heart failure (Failing RMC). The y-axis is in units of ng/mL, and

runs from 0 to 20 in increments of 2. * = $p < 0.05$ compared to BM MSC. ** = $p < 0.01$ compared to Normal RMC.

[0022] **FIG. 5A** is a graph showing the effect of IGF-1 supplementation on cardiac alternans in RMCs obtained from human hearts with heart failure (Failing RMC). The left bar is for the failing RMCs only. The right bar is failing RMCs supplemented with IGF-1 (50 ng/mL). The y-axis is in arbitrary units, and runs from 0 to 0.7 in increments of 0.1. * = $p < 0.0001$.

[0023] **FIG. 5B** is a graph showing the effect of IGF-1 supplementation on action potential duration in RMCs obtained from human hearts with heart failure (Failing RMC). The left bar is for the failing RMCs only. The right bar is failing RMCs supplemented with IGF-1 (50 ng/mL). The y-axis is in milliseconds (msec), and runs from 900 to 1250 in increments of 50. * = $p < 0.02$.

[0024] **FIG. 6** is a graph showing Ca^{2+} alternans measured in human cardiac myocytes alone (H_2O_2+hCM), co-cultured with RMCs obtained from human hearts without heart failure (+nonFailing RMC), co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC). All cells were exposed to H_2O_2. The y-axis is in arbitrary units, and runs from 0 to 100 in increments of 20. $p = 0.005$.

[0025] **FIG. 7A** is a bar graph showing Ca^{2+} alternans measured when human cardiac myocytes exposed to H_2O_2 were incubated with various compounds. From left to right, the bars are hCM alone as the control (H_2O_2+hCM); hCM co-cultured with RMCs obtained from human hearts without heart failure (+nonFailing RMC); hCM exposed to 50 ng/mL IGF-1 (+IGF); hCM exposed to 50 ng/mL VEGF (+VEGF); hCM exposed to 50ng/mL IGF-1 and 100 nmol/L of wortmannin (+IGF+WORT); and hCM co-cultured with RMCs obtained from human hearts with heart failure and exposed to 50 ng/mL IGF-1 (+Failing RMC+IGF). All cells were exposed to H_2O_2. The y-axis is in arbitrary units, and runs from 0 to 90 in increments of 10. Significant differences are indicated by *.

[0026] **FIG. 7B** is a bar graph showing ELISA results for IGF-1 in four different cell populations. Those populations are bone marrow mesenchymal stem cells (BM MSC); normal resident mesenchymal cells (RMCs) obtained from human hearts without heart failure (nonFailing RMC); RMCs obtained from human hearts with heart failure (Failing

RMC); and human cardiac myocytes (hCM). The y-axis is in ng/mL, and runs from 0 to 20 in increments of 5. Significant differences are indicated by *.

[0027] **FIG. 7C** is a bar graph showing results for IGF-1 when simulating co-culture conditions where the level for hCM was added to the nonFailing RMC and Failing RMC levels. The y-axis is in ng/mL, and runs from 0 to 14 in increments of 2. Significant differences are indicated by *.

[0028] **FIG. 8A** is a bar graph showing Ca^{2+} alternans measured under normal conditions (no H_2O_2) for hCM alone; co-cultured with RMCs obtained from human hearts without heart failure (+nonFailing RMC); and co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC). The y-axis is in arbitrary units, and runs from 0 to 100 in increments of 20.

[0029] **FIG. 8B** is a bar graph showing APD50 measured under normal conditions (no H_2O_2) for hCM alone; co-cultured with RMCs obtained from human hearts without heart failure (+nonFailing RMC); and co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC). The y-axis is in milliseconds, and runs from 500 to 1000 in increments of 100.

[0030] **FIG. 8C** is a bar graph showing APD90 measured under normal conditions (no H_2O_2) for hCM alone; co-cultured with RMCs obtained from human hearts without heart failure (+nonFailing RMC); and co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC). The y-axis is in milliseconds, and runs from 900 to 1400 in increments of 100.

[0031] **FIG. 9A** is a bar graph showing the effect of IL-1β and IL-6 on Ca^{2+} alternans. The bars are, from left to right, for hCM alone; hCM co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC); hCM exposed to 40 ng/mL IL-1β (+IL1B); hCM exposed to 20 ng/mL IL-6 (+IL6); and hCM co-cultured with RMCs obtained from human hearts with heart failure and exposed to 50 ng/mL IGF-1 (+Failing RMC+IGF). The y-axis is in arbitrary units, and runs from 0 to 100 in increments of 10.

[0032] **FIG. 9B** is a bar graph showing the effect of IL-1β and IL-6 on APD50. The bars are, from left to right, for hCM alone; hCM co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC); hCM exposed to 40 ng/mL IL-1β (+IL1B); hCM exposed to 20 ng/mL IL-6 (+IL6); and hCM co-cultured with RMCs obtained from

human hearts with heart failure and exposed to 50 ng/mL IGF-1 (+Failing RMC+IGF). The y-axis is in millseconds, and runs from 500 to 900 in increments of 100.

[0033] **FIG. 9C** is a bar graph showing the effect of IL-1β and IL-6 on APD90. The bars are, from left to right, for hCM alone; hCM co-cultured with RMCs obtained from human hearts with heart failure (+Failing RMC); hCM exposed to 40 ng/mL IL-1β (+IL1B); hCM exposed to 20 ng/mL IL-6 (+IL6); and hCM co-cultured with RMCs obtained from human hearts with heart failure and exposed to 50 ng/mL IGF-1 (+Failing RMC+IGF). The y-axis is in milliseconds, and runs from 700 to 1200 in increments of 100.

[0034] **FIG. 9D** is a bar graph showing ELISA results for IL-1β in four different cell populations. Those populations are bone marrow mesenchymal stem cells (BM MSC); normal resident mesenchymal cells (RMCs) obtained from human hearts without heart failure (nonFailing RMC); RMCs obtained from human hearts with heart failure (Failing RMC); and human cardiac myocytes (hCM). The y-axis is in pg/mL, and runs from 0 to 600 in increments of 100. Significant differences are indicated by *.

[0035] **FIG. 9E** is a bar graph showing ELISA results for IL-6 in four different cell populations. Those populations are bone marrow mesenchymal stem cells (BM MSC); normal resident mesenchymal cells (RMCs) obtained from human hearts without heart failure (nonFailing RMC); RMCs obtained from human hearts with heart failure (Failing RMC); and human cardiac myocytes (hCM). The y-axis is in ng/mL, and runs from 0 to 20 in increments of 50. Significant differences are indicated by *.

DETAILED DESCRIPTION

[0036] The present disclosure may be understood more readily by reference to the following detailed description of desired embodiments. In the following specification and the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings.

[0037] Although specific terms are used in the following description for the sake of clarity, these terms are intended to refer only to the particular structure of the embodiments selected for illustration in the drawings, and are not intended to define or limit the scope of the disclosure. In the drawings and the following description below, it is to be understood that like numeric designations refer to components of like function.

[0038] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0039] As used in the specification and in the claims, the open-ended transitional phrases “comprise(s),” “include(s),” “having,” “contain(s),” and variants thereof require the presence of the named components/steps and permit the presence of other components/steps. These phrases should also be construed as disclosing the closed-ended phrases "consist of" or "consist essentially of" that permit only the named components/steps and unavoidable impurities, and exclude other components/steps.

[0040] Numerical values in the specification and claims of this application should be understood to include numerical values which are the same when reduced to the same number of significant figures and numerical values which differ from the stated value by less than the experimental error of conventional measurement technique of the type described in the present application to determine the value.

[0041] All ranges disclosed herein are inclusive of the recited endpoint and independently combinable (for example, the range of “from 2 grams to 10 grams” is inclusive of the endpoints, 2 grams and 10 grams, and all the intermediate values).

[0042] The term “about” can be used to include any numerical value that can vary without changing the basic function of that value. When used with a range, “about” also discloses the range defined by the absolute values of the two endpoints, e.g. “about 2 to about 4” also discloses the range “from 2 to 4.” The term “about” may refer to plus or minus 10% of the indicated number.

[0043] The term "patient" or "subject" is used throughout the specification to describe an animal, generally a mammal and preferably a human, to whom treatment, including IGF-1 treatment, and variations thereof according to the present disclosure is provided.

[0044] The term "effective amount" is used herein, unless otherwise indicated, to describe an amount of a compound or composition or component which, in context, is used to produce or effect an intended result when administered as a single dosage.

[0045] The terms "prevent” or “prevention" are used to mean "reducing the likelihood" of a condition or disease state from occurring. These terms are qualitative, and can refer to a single patient or a population of patients.

[0046] Insulin-like Growth Factor-1 ("IGF-1"), also known as somatomedin, is a protein that is similar in molecular structure to insulin. IGF-1 consists of 70 amino acids in a single chain with three disulfide bonds, and has a molecular weight of 7,649 daltons.

[0047] Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidants within the body, and particularly the heart in the present disclosure. Oxidative stress is linked to an increase in ROS and a decrease in the antioxidant reserve, which is a defense mechanism in cardiac muscle cells ("cardiomyocytes"). Increased formation of ROS indicates the presence of oxidative stress, which can play a role in facilitating a cardiac rhythm disturbance. ROS can affect all major ionic currents in the heart's electrical system. Specifically, increased amounts of ROS affects several ionic (e.g., Na^+) currents in the cardiomyocytes, alters intracellular handling of Ca^{2+}, and/or affects cardiac potassium channels. It is believed that these effects of ROS can potentially decrease the repolarization reserve, which triggers arrhythmia.

[0048] The present disclosure relates to methods for the treatment of cardiac arrhythmia of a patient in need thereof. This extends to preventive therapies intended to prevent arrhythmia, where the treatment is performed on a patient at risk for developing arrhythmia and/or before the patient exhibits arrhythmia. The scope also extends to antiarrhythmic therapies intended to reduce or stop arrhythmia that is already diagnosed in the patient, where the patient is showing irregular heartbeats. Similarly, a patient "in need thereof" can be a subject who exhibits one or more symptoms or indicia of arrhythmia.

[0049] Initially, one or more diagnostic procedures are performed to identify, detect, or diagnose cardiac arrhythmia in the subject. In some embodiments, a non-invasive approach is used to identify electrical rhythms in the heart. For example, an electrical rhythm abnormality can be detected in the patient using an electrocardiogram, or a similar medical device that measures electrical function. Examples of such abnormalities include a diagnosis of cardiac alternans, ventricular tachycardia, or ventricular fibrillation, or other ventricular arrhythmia, or atrial fibrillation. Other methods that predict high arrhythmia risk (e.g. family genetic history) could also be used to identify a patient in need of treatment.

[0050] In some other embodiments, a biological sample is collected from the patient and a test is performed on the biological sample to diagnose oxidative stress. The test could measure levels of ROS for comparison to a baseline reference. In response to an increased ROS level relative to a threshold value, the patient can be diagnosed as having arrhythmia or being at risk for arrhythmia. Alternatively, the test can measure levels of antioxidants for comparison to a baseline reference. The antioxidants can include molecular compounds and enzymes. In response to a decreased antioxidant level relative to a threshold value, the patient can be diagnosed as having arrhythmia or being at risk for arrhythmia. Other proteins or biomolecules that correlate with oxidative stress can also be tested for. IGF-1 levels, the levels of IGF-1 binding proteins, or the levels of IGF-1 receptors in the biological sample can also be directly measured.

[0051] The biological sample can be any biological specimen, such as, blood, urine, or other fluids. In some embodiments, the biological sample is a blood sample. In other embodiments, a living biological sample of cells can be collected from the body, and more particularly from the heart muscle, using a biopsy. Cardiac tissue contains numerous cell types.

[0052] There is no limitation made herein on the process used to determine a marker of oxidative stress in the patient. There is furthermore no limitation made herein on the marker used to indicate oxidative stress in the patient. In response to the marker indicating oxidative stress in the patient, the patient is determined to have oxidative stress, be or be at risk of having arrhythmia, and is therefore deemed a candidate for the IGF-1 therapy disclosed herein.

[0053] After being identified as being in need of treatment, a therapeutically effective amount of IGF-1 or an analog thereof is administered to the patient. The IGF-1 or the analog of IGF-1 can be administered to the patient in a sufficient quantity to reduce the measure of oxidative stress, or the effects of oxidative stress, in the patient.

[0054] The IGF-1 or the analog of IGF-1 can be administered intramuscularly, intravenously, intracoronary, intraperitoneally, or parentally. The term “analog” is used to refer to structural analogs (i.e. the analog is also a protein). In particular, the analog should bind to the IGF-1 receptor. It is specifically contemplated that one analog which

can be used in the present disclosure is PEGylated IGF-1. The IGF-1 can be obtained from a natural source, or can be synthetically produced.

[0055] The present disclosure also contemplates the use / administration of a synthetic drug that mimics the active portion of the IGF-1 molecule, which may be produced with a more favorable benefit/risk profile. These drugs are contemplated for specifically targeting the antiarrhythmic pathways and for avoiding other non-beneficial pathways.

[0056] One aspect of the present disclosure is using IGF-1, a naturally occurring hormone in humans, as therapy for cardiac arrhythmias. Using IGF-1 directly for therapy overcomes several limitations associated with the implementation of stem cells (known to excrete IGF-1) for therapy. An additional aspect of using the IGF-1 as an antiarrhythmic agent herein is a reduced risk or prevention of several other cardiac diseases and outcomes associated with arrhythmia. Another aspect of IGF-1, as a treatment for preventing arrhythmias, provides important immediate insight for the mechanisms of arrhythmias in general.

[0057] Experiments indicate that IGF-1 reduces cardiac arrhythmias by working through the PI3Kinase pathway. It is thus contemplated that administering agents that activate / upregulate the PI3Kinase pathway may also have the same therapeutic benefit with respect to cardiac arrhythmia. Any agent that activates or upregulates the PI3Kinase pathway could be administered. Examples of such agents might include growth hormone; or IGF-2; or FOX01 transcription factor (also known as Forkhead box protein O1, FKH1, FKHR, or FOXO1A). The PI3K activating agent may be a protein or a molecule. The PI3K pathway is naturally downregulated by a protein known as phosphatase and tensin homolog (PTEN). Any drug which downregulates PTEN would result in upregulating of the PI3K pathway, and would be considered a PI3K activating agent as well. In addition, there are eight IGF binding proteins (known as IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, IGFBP7, and IGFALS) that sequester IGF-1 or IGF-2 in the blood stream. This sequestration results in decreased free IGF in circulation in the blood, and thus decreased PI3K pathway activation. Thus, administration of anti-IGFBP agents, such as antibodies that bind to IGFBP (i.e. compete with IGF for binding to any IGFBP), should result in a release of bound IGF, increasing free or active IGF in circulation and an increase in PI3K pathway activation. Such anti-IGFBP agents should thus be

considered a PI3K activating agent as well. Combinations of IGF-1 with an anti-IGFBP agent are expressly contemplated. Any agent that causes cells to overexpress / oversecrete IGF would also be considered a PI3K activating agent.

[0058] The present disclosure will further be illustrated in the following non-limiting examples, it being understood that these examples are intended to be illustrative only and that the disclosure is not intended to be limited to the materials, conditions, process parameters and the like recited herein.

FIRST SET OF EXAMPLES

[0059] Previous studies have shown that when human mesenchymal stem cells (hMSCs) are co-cultured with human cardiac myocytes (hCMs) *in vitro*, cardiac alternans is suppressed. This is illustrated in **FIG. 2A** and **FIG. 2B**. **FIG. 2A** shows electrical traces for hCMs alone (top) and hCMs co-cultured with hMSCs (bottom). On the top, the hCMs alone show Ca^{2+} transient recordings that exhibit cardiac alternans, manifested as a beat-to-beat oscillation of the Ca^{2+} transient amplitude (red arrows for beat a and b). When hCMs are co-cultured with hMSCs, Ca^{2+} transient alternans is suppressed (bottom trace). **FIG. 2B** is a graph showing that hMSCs can significantly decrease cardiac alternans, as manifested in Ca^{2+} alternans (Ca^{2+} ALT). It was also previously determined that this effect is due to activation of a PI3K-mediated nitroso-redox pathway that improves Ca^{2+} regulation.

[0060] Experiments were performed to determine if IGF-1 accounted for the suppression of cardiac alternans. Human cardiac myocytes (hCMs) were exposed to H2O2 (a reactive oxygen species) to simulate disease conditions. The magnitude of Ca^{2+} alternans (Ca^{2+} ALT) was then measured in the presence of IGF-1 (50 ng/mL) and VEGF (50 ng/mL). In the presence of IGF-1, Ca^{2+} ALT was significantly decreased. In contrast, VEGF had no significant effect on Ca^{2+} ALT. These results are shown in **FIG. 3**.

[0061] Next, secreted IGF-1 levels were measured in hMSC-like cells (called resident mesenchymal cells or RMC) isolated from human hearts with heart failure (failing RMCs), RMCs isolated from human hearts without heart failure (normal RMCs), and hMSCs. These results are shown in **FIG. 4**, and show that cells that suppressed Ca^{2+} alternans (hMSCs, normal RMCs) had much higher levels of IGF-1 compared to cells that did not suppress Ca^{2+} alternans (failing RMCs).

[0062] Finally, failing RMCs were supplemented with IGF-1 to determine if this could rescue the functional effects. **FIG. 5A** and **FIG. 5B** are graphs showing the effect of failing RMCs with and without IGF-1 supplementation (50 ng/mL) on Ca^{2+} alternans and action potential duration (APD). These graphs show that when failing RMCs are supplemented with IGF-1, Ca^{2+} alternans and APD are decreased.

[0063] Taken together, these results suggest that administering IGF-1 will suppress cardiac alternans.

SECOND SET OF EXAMPLES

[0064] Human cardiac myocytes (hCMs) derived from induced pluripotent stem cells were purchased from Cellular Dynamics Inc. Cell pellets in the cryoprecipitate tube were thawed and cultured as monolayers according to the protocol provided by the manufacturer. Cells were plated onto fibronectin coated Biolite 96-well plates (Catalog #130188, ThermoFisher Scientific, Waltham, Massachusetts) prior to experimentation at 1.0×10^4, 3.3×10^4, or 6.6×10^4 cells per well, corresponding to cell densities of 3.1×10^2, 1.0×10^3 and 2.0×10^3 cells/mm^2, respectively. Culture media was changed every 2 days, until day 14-20 when experiments were performed.

[0065] Two different types of resident mesenchymal cells (RMC) were used. One type was isolated from human hearts with heart failure (failing RMCs). The second type was isolated from human hearts without heart failure (normal RMCs). The human heart tissue was washed with PBS, minced into tiny pieces, extensively washed with PBS again, and digested with 0.2% Collagenase Type 2 for 3 hours in the incubator. Intermittent vigorous pipetting was done to aid digestion and isolate cells along with constant monitoring under the microscope. Digested tissue with isolated cells was centrifuged, collagenase was removed, and plated onto 35mm plastic dish with RMC isolation media containing F12/DMEM (Gibco), E8, MEM/NEA (Non-essential amino acids), FGF Basic (R&D systems) 20 ng/ml, 20% FBS (Biowest), and Penicillin/Streptomycin. This media was replaced every 3-4 days. On day 2-3, unattached cells and debris were washed with PBS and attached stromal cells were allowed to proliferate. This washing selects for only plastic adherent cells like RMCs and removes other cell types.

[0066] After 8-10 days and appropriate proliferation in the RMC isolation medium, cells were passed, and further proliferated and maintained in the RMC maintenance medium. RMC maintenance medium consists of DMEM Low Glucose (Gibco), MEM/NEA (Non-essential amino acids), FGF Basic (R&D systems) 10 ng/ml, 10% FBS (Biowest), and Penicillin/Streptomycin.

[0067] After RMCs were 70-80% confluent, they were cultured in the adipocyte differentiation media, DM-2 (Zenbio), for 1-2 weeks, and then adipocyte maintenance media, AM-1 (Zenbio), for 1-2 weeks per the manufacturer instructions. DM-2 contains

insulin, dexamethasone, IBMX, PPAR-gamma agonist. AM-1 contains insulin and dexamethasone. Adipose vacuoles could be noted within the RMCs as early as 1 week into differentiation. Adipocytes were confirmed by staining with Oil Red-O per the protocol in the lipid staining kit, ST-R100 (Zenbio).

[0068] Osteogenesis was induced by exposing stromal cells that are 70-80% confluent to osteogenesis media for 4 weeks with media changes every 3-4 days. Osteogenesis media contains DMEM High Glucose (Sigma), FBS (Sigma), Dexamethasone (Sigma), Penicillin/Streptomycin, Ascorbic acid 2-phosphate (Wako USA), B-glycerophosphate (Calbiochem), and BMP-2.

[0069] Human bone marrow mesenchymal stem cells (BM MSCs) were stored at a temperature of -80°C. For use, they were thawed, cultured, proliferated and maintained with a Stromal Cell Maintenance Medium that contains DMEM Low glucose (Gibco), Non-Essential Amino acids (NEA), 10% FBS (Biowest), and Penicillin/Streptomycin. Cells were passed 3-5 times prior to use.

[0070] Monolayers were formed by hCMs plated at 50 $x10^4$ cells per 25mm coverslip coated with fibronectin for each functional experiment. RMCs (passage 3-5) were plated onto the coverslips with matured hCMs at a density of 15 x 10^4 cells per 25mm coverslip and maintained in hCM media for 2 days prior to functional measurements. For experiments with factor supplementation, all factors used were human recombinant factors from Sinobiological. Each factor (IGF-1 (50 ng/ml), VEGF (50 ng/ml), IL-1B (40 ng/ml), IL-6 (20 ng/ml)) was maintained in culture for 48 hrs prior to and during experimentation. For assessing if IGF-1 induced the PI3K signaling pathway, a PI3K inhibitor, Wortmannin (100 nmol/L), was incubated together with IGF-1.

[0071] Alternans experiments and analysis were performed with calcium staining and imaging using Fluo4 (Sigma). Alternans was measured at a pacing period of 800 milliseconds (ms) at room temperature. Response to oxidative stress was measured by repeating the same measurement 2 minutes after administration of H_2O_2 (200 μM) to the same cells. Mean % alternans was determined for each monolayer. Action potentials were measured with FluoVolt imaging (Sigma) after staining with voltage sensitive dye at room temperature. Action potential durations (APD50 and APD90) were measured at room temperature when cells were paced at a period of 2000 ms. Spontaneous calcium

release (SCR) activity was measured via Fluo4 calcium imaging, immediately after stopping rapid pacing (periods: 1200 ms, 1000 ms, 800 ms, 600 ms, 400 ms, then stop pacing) post H_2O_2 administration. The presence of SCRs was identified by an expert blinded to the groups.

[0072] It was previously shown that bone marrow derived MSCs can suppress cardiac alternans measured in hCM monolayers exposed to oxidative stress (to simulate disease conditions). It was desired to learn whether the same is true for RMCs isolated from non-failing and failing hearts. **FIG. 6** shows Ca^{2+} alternans (beta-to-beat amplitude oscillation) measured in hCM monolayers when exposed to H_2O_2 alone and when cultured alone or co-cultured with non-failing or failing RMCs. Compared to the control (hCM only), RMCs from non-failing hearts decreased Ca^{2+} alternans, whereas RMCs from failing hearts had no effect under these conditions. Filled circles indicate RMCs from living persons, and empty circles indicate RMCs obtained from donor hearts. The suppression of alternans by non-failing RMCs from donor hearts was greater than that from living persons. However, this difference could not account for the significant differences observed when the samples were pooled.

[0073] As discussed above, the suppression of Ca^{2+} alternans by bone marrow MSCs could be attributed to activation of the PI3K-Akt pathway. To further determine the exact signaling mechanisms involved, two PI3K-Akt activators, IGF-1 and VEGF, in the absence of RMCs. Shown in **FIG. 7A** are the average magnitude of Ca^{2+} alternans when hCMs exposed to H_2O_2 were incubated with IGF, VEGF, IGF+WORT, and failing RMCs+IGF. IGF-1 significantly suppressed alternans just as non-failing RMCs did, but VEGF did not. When wortmannin (WORT), an inhibitor of the PI3K-Akt pathway, was incubated with IGF-1, the suppression of alternans was lost. Finally, when RMCs from failing hearts were incubated with IGF, the suppression of alternans was rescued. Levels of IGF were tested in all three individual cell populations, and are shown in **FIG. 7B**. IGF-1 was significantly lower in failing RMCs compared to non-failing RMCs. If the levels of IGF from separate populations are added with that from hCM to simulate co-culture conditions, the total IGF levels mirror the action on Ca2+ alternans (**Fig. 7C**). These results suggest that IGF can explain the effects of non-failing and failing RMCs on Ca^{2+} alternans.

[0074] Heart failure is associated with numerous electrophysiological substrates, including Ca^{2+} alternans and prolongation of APD. Given that non-myocytes in the heart are known to secrete harmful cytokines, it is possible that RMCs from failing hearts can account for some of the electrophysiological substrates observed in heart failure. The effects of non-failing and failing RMCs on Ca^{2+} alternans and APD in the absence of oxidative stress are shown in **FIGS. 8A-8C**. Failing RMCs increase Ca^{2+} alternans (**FIG. 8A**), APD50 (**FIG. 8B**), and APD90 (**FIG. 8C**) compared to hCM alone and non-failing RMCs. Filled circles indicate RMCs from living persons, and empty circles indicate RMCs obtained from donor hearts. In **FIG. 8B**, the increase in APD50 with failing RMCs was greater for donor hearts compared to living persons; however, this difference could not account for the significant differences observed when the samples were pooled.

[0075] Spontaneous calcium releases (SCRs) are typically associated with Ca^{2+} dysregulation and heart failure. In the absence of H_2O_2, SCRs were rarely observed in hCM monolayers when cultured alone or when co-cultured with bone marrow MSCs, non-failing RMCs, and failing RMCs. However, in the presence of H_2O_2 and following rapid pacing, SCRs were observed when hCM were cultured with failing RMCs but not with non-failing RMCs. In 14 experiments with non-failing RMC co-cultures, no SCRs were observed. In contrast, seven (7) out of 19 experiments exhibited SCR activity in failing RMC co-cultures ($p < 0.02$).

[0076] To better understand the signals responsible for these electrophysiological substrates observed, two cytokines known to be harmful and secreted by non-myocytes in the heart were investigated. **FIGS. 9A-9C** show the effect of IL-1β and IL-6 on Ca^{2+} alternans (**FIG. 9A**), APD50 (**FIG. 9B**), and APD90 (**FIG. 9C**). Interestingly, as seen in **FIG. 9A**, IL-1β significantly decreased Ca^{2+} alternans compared to hCM alone, whereas IL-6 significantly increased Ca^{2+} alternans. In contrast, as seen in **FIG. 9B** and **FIG. 9C**, both IL-1β and IL-6 significantly increased APD50 and APD90 compared to hCM alone, similar to the effect of failing RMCs.

[0077] **FIG. 9D** shows the levels of IL-1β in each cell population. **FIG. 9E** shows the levels of IL-6 in each cell population. Both IL-1β and IL-6 (Panel B) were significantly increased in failing RMCs compared to non-failing RMCs and bone marrow MSCs. Levels of IL-1β and IL-6 in hCM were very low and, thus, unlikely to explain the increase

in Ca^{2+} alternans and APD. These results suggest that increased levels of IL-1β and IL-6 may explain the increase in APD; however, they have an opposite effect on Ca^{2+} alternans where IL-6 alone seems to be responsible.

[0078] The main findings are that under oxidative stress conditions, RMCs from non-failing hearts suppress Ca^{2+} alternans and RMCs from failing hearts do not. These effects appear to be mediated by IGF-1. Furthermore, under normal conditions, non-failing RMCs have no effect on Ca^{2+} alternans or APD. However, failing RMCs increased Ca^{2+} alternans and APD. In this case, the cytokines IL-1β and IL-6 may be responsible.

[0079] Our results suggest that IGF-1 is responsible for the suppression of Ca^{2+} alternans that was associated with non-failing RMCs. Furthermore, IGF-1 levels were much higher in non-failing RMCs compared to failing RMCs, which were unable to suppress Ca^{2+} alternans. Finally, wortmannin, a PI3K inhibitor, blocked the effect of non-failing RMCs, and IGF-1 supplementation was able to rescue failing RMCs.

[0080] The present disclosure has been described with reference to exemplary embodiments. Modifications and alterations will occur to others upon reading and understanding the preceding detailed description. It is intended that the present disclosure be construed as including all such modifications and alterations insofar as they come within the scope of the appended claims or the equivalents thereof.

CLAIMS:

1. A method for the treatment of cardiac arrhythmia or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of IGF-1 or an analog thereof.

2. The method of claim 1, wherein the patient is identified by a finding of cardiac alternans in an electrocardiogram of the patient.

3. The method of claim 1, wherein the patient is identified by a finding of ventricular tachycardia in the patient.

4. The method of claim 1, wherein the patient is identified by a finding of ventricular fibrillation or other ventricular arrhythmia in the patient.

5. The method of claim 1, wherein the patient is identified by finding a level of IGF-1 or a marker of oxidative stress in a biological sample obtained from the patient and comparing the level of the IGF-1 or the marker of oxidative stress to a threshold value.

6. The method of claim 5, wherein the biological sample is blood,

7. The method of claim 5, wherein the marker of oxidative stress is a reactive oxygen species (ROS), or a redox molecule that counterbalances a ROS; or a protein or a biomolecule.

8. The method of claim 1, wherein the IGF-1 or analog thereof is administered intramuscularly, intravenously, intracoronary, intraperitoneally, parentally, or orally.

9. The method of claim 1, wherein the IGF-1 or analog thereof is PEGylated.

10. A method for the treatment of cardiac alternans or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of IGF-1 or an analog thereof.

11. The method of claim 10, wherein the patient is identified by a finding of cardiac alternans in an electrocardiogram of the patient.

12. The method of claim 10, wherein the patient is identified by a finding of ventricular tachycardia in the patient.

13. The method of claim 10, wherein the patient is identified by a finding of ventricular fibrillation or other ventricular arrhythmia in the patient.

14. The method of claim 10, wherein the patient is identified by finding a level of IGF-1 or a marker of oxidative stress in a biological sample obtained from the patient and comparing the level of the IGF-1 or the marker of oxidative stress to a threshold value.

15. The method of claim 14, wherein the biological sample is blood,

16. The method of claim 14, wherein the marker of oxidative stress is a reactive oxygen species (ROS), or a redox molecule that counterbalances a ROS; or a protein or a biomolecule.

17. The method of claim 10, wherein the IGF-1 or analog thereof is administered intramuscularly, intravenously, intracoronary, intraperitoneally, parentally, or orally.

18. The method of claim 10, wherein the IGF-1 or analog thereof is PEGylated.

19. A method for the treatment of cardiac arrhythmia or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of an IGF-1 mimetic.

20. A method for the treatment of cardiac arrhythmia or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of a PI3K activating agent.

21. A method for the treatment of cardiac alternans or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of a PI3K activating agent.

22. A method for the treatment of cardiac arrhythmia or action potential duration in a patient, comprising:

administering to the patient a therapeutically effective amount of a PI3K activating agent.

23. The method of any of claims 20-22, wherein the PI3K activating agent is IGF-1 or an analog thereof.

24. The method of any of claims 20-22, wherein the PI3K activating agent is growth hormone; or IGF-2; or FOX01 transcription factor; or an anti-IGFBP agent; or a PTEN downregulator.

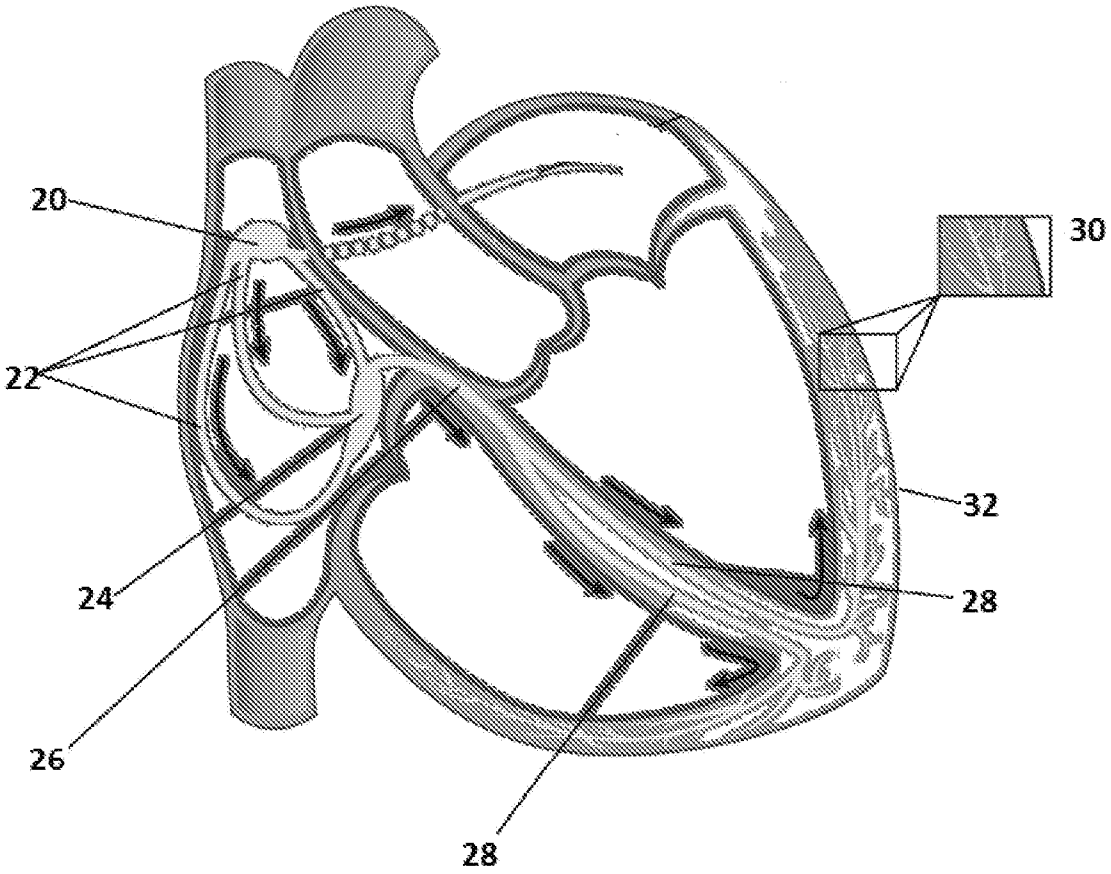

FIG. 1

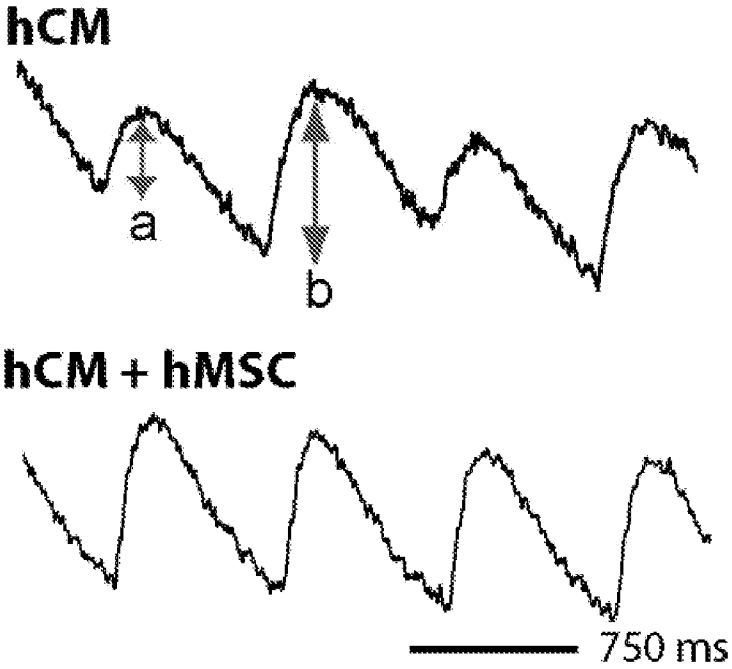
hCM
a
b
hCM + hMSC
750 ms

FIG. 2A

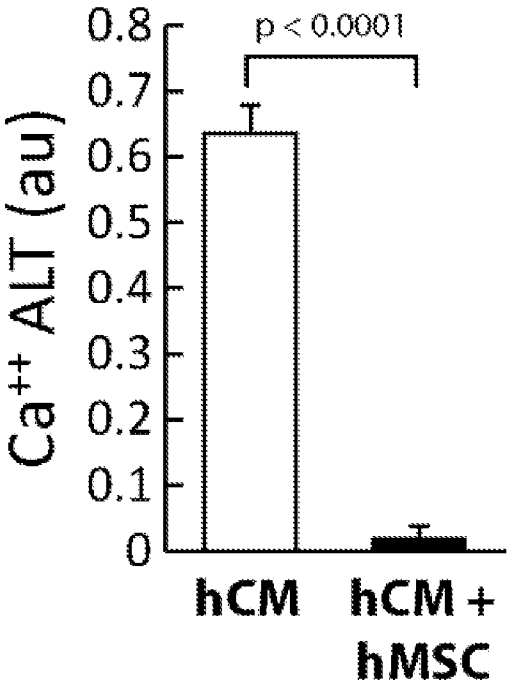
p < 0.0001
Ca++ ALT (au)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
hCM
hCM + hMSC

FIG. 2B

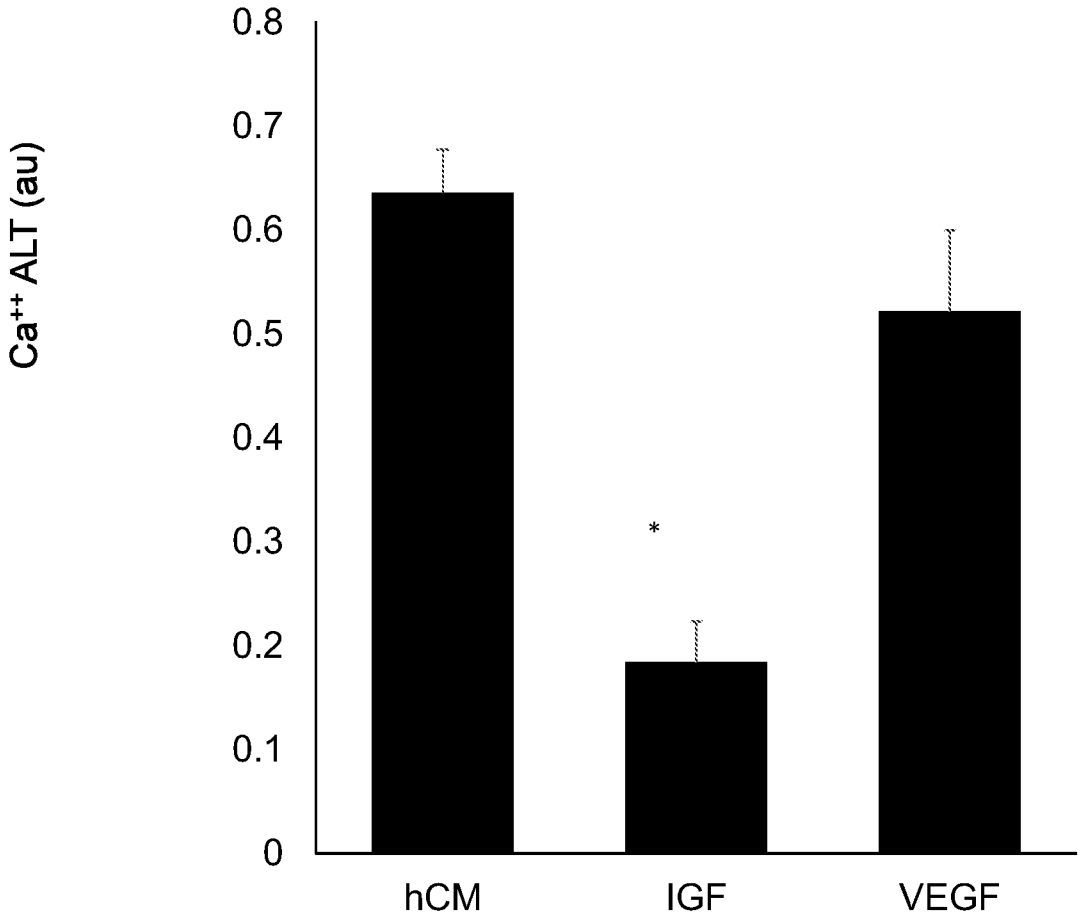

FIG. 3

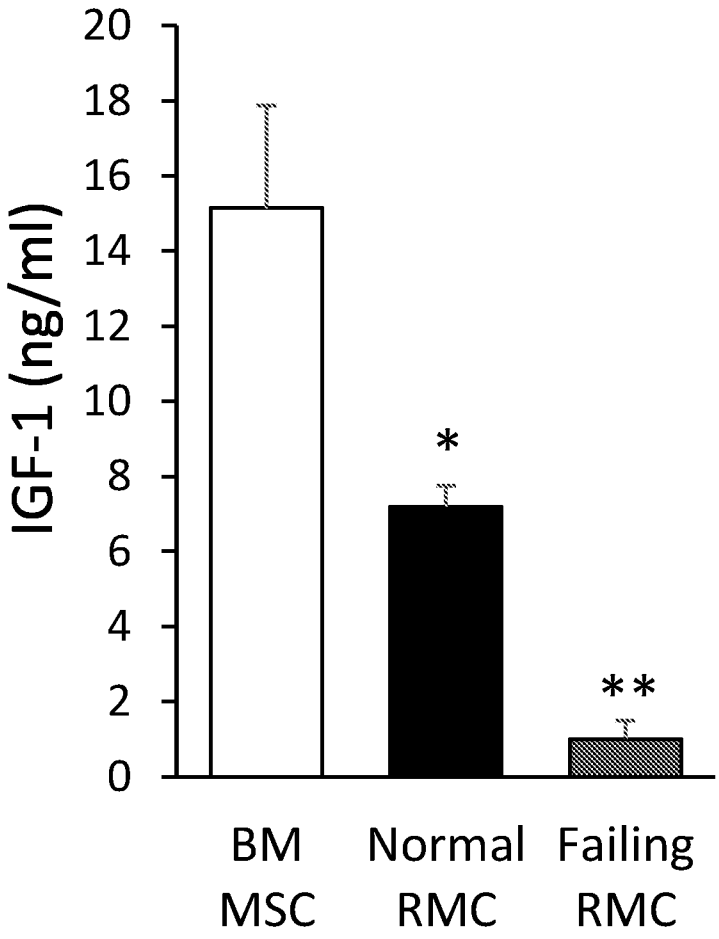

FIG. 4

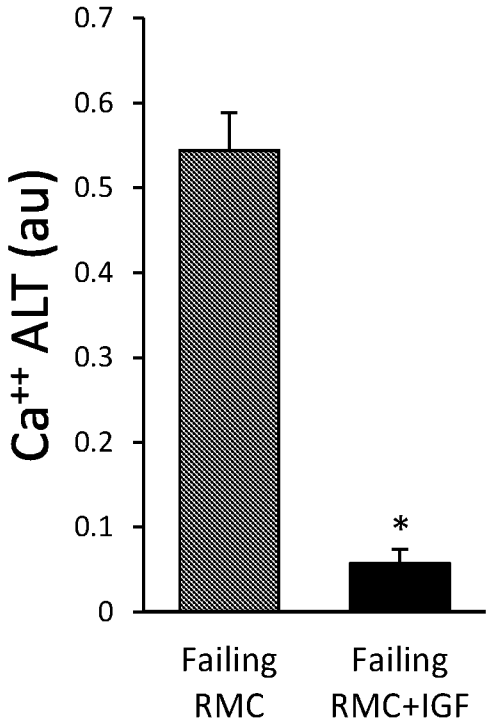
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ca++ ALT (au)
*
Failing RMC
Failing RMC+IGF

FIG. 5A

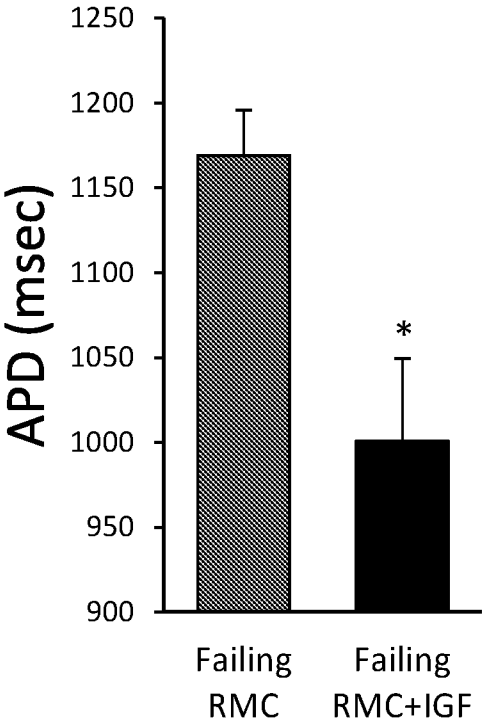
1250
1200
1150
1100
1050
1000
950
900
APD (msec)
*
Failing RMC
Failing RMC+IGF

FIG. 5B

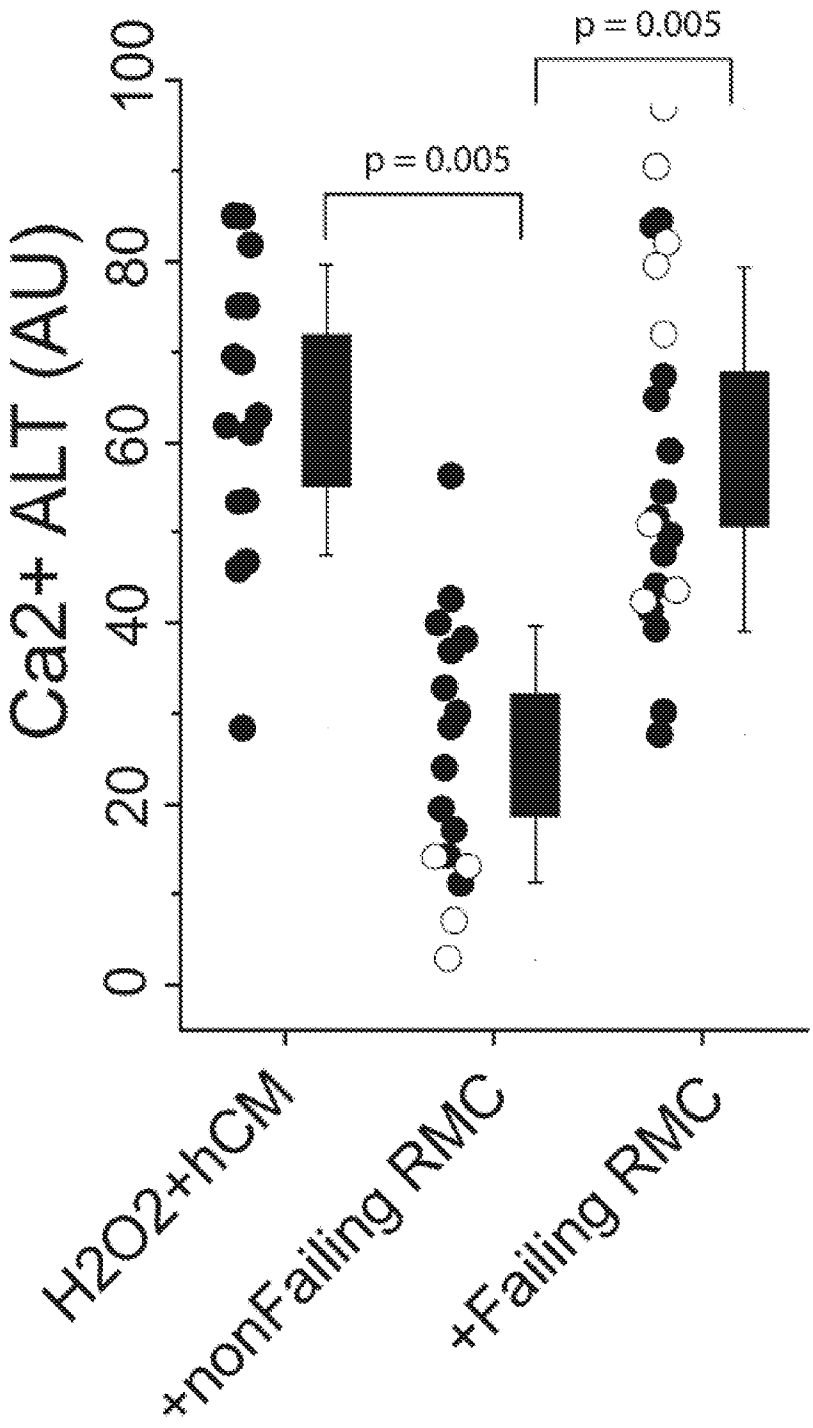
p = 0.005
p = 0.005
Ca2+ ALT (AU)
0
20
40
60
80
100
H2O2+hCM
+nonFailing RMC
+Failing RMC

FIG. 6

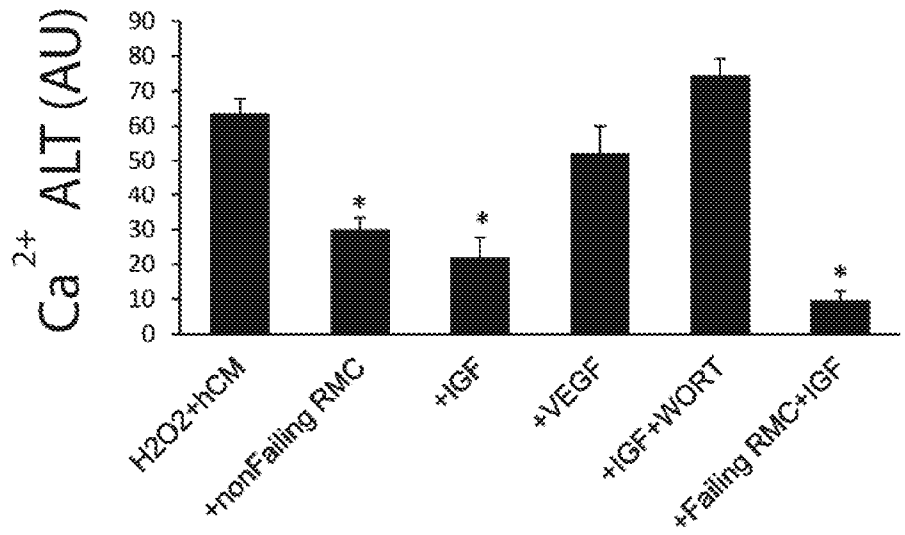

FIG. 7A

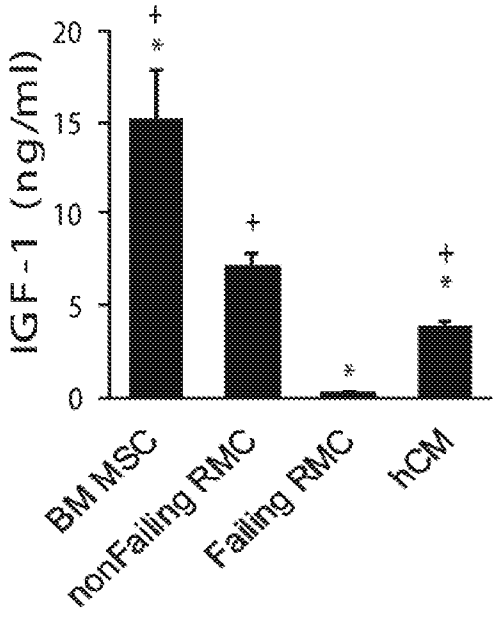

FIG. 7B

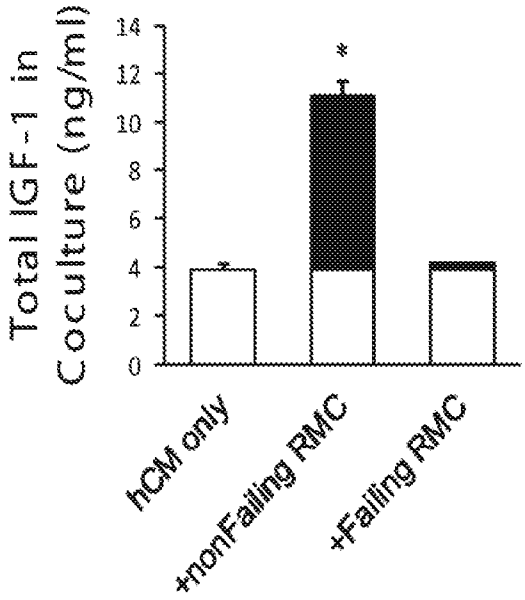

FIG. 7C

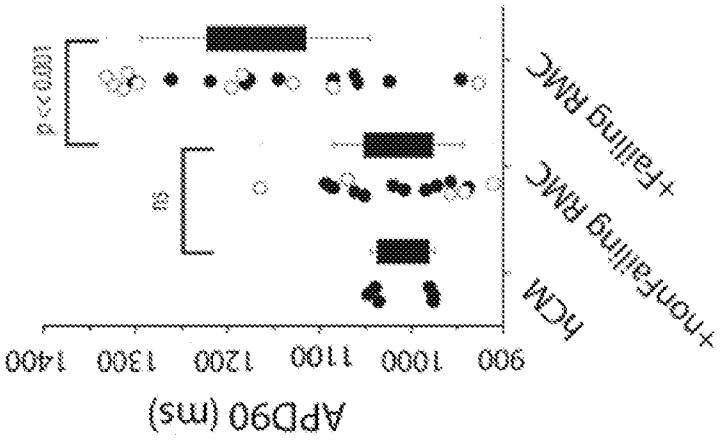

FIG. 8C

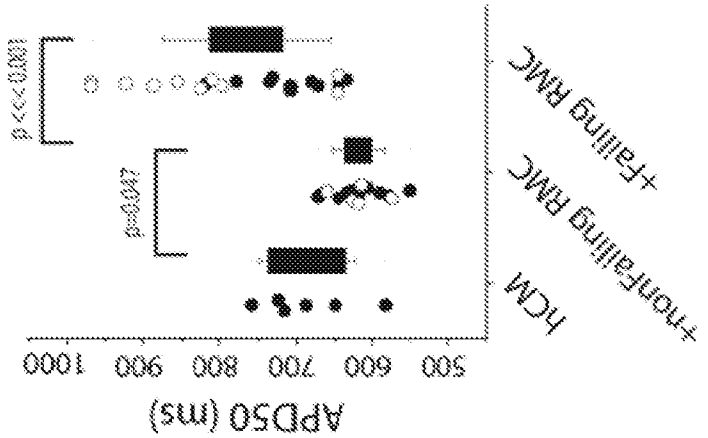

FIG. 8B

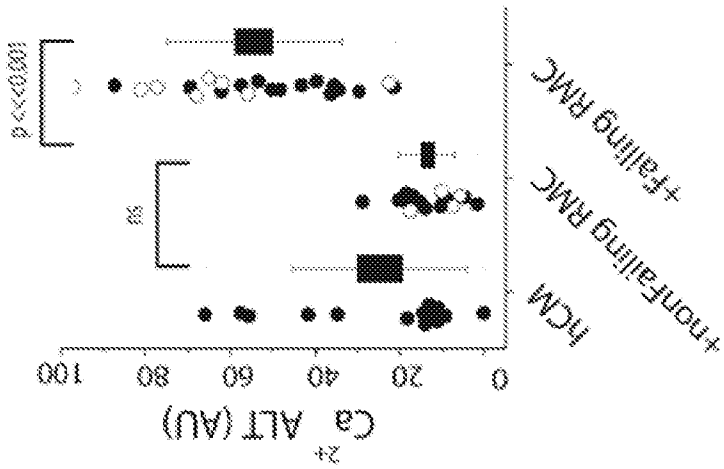

FIG. 8A

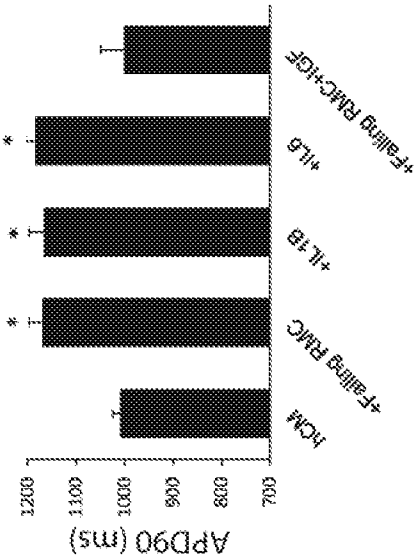

FIG. 9C

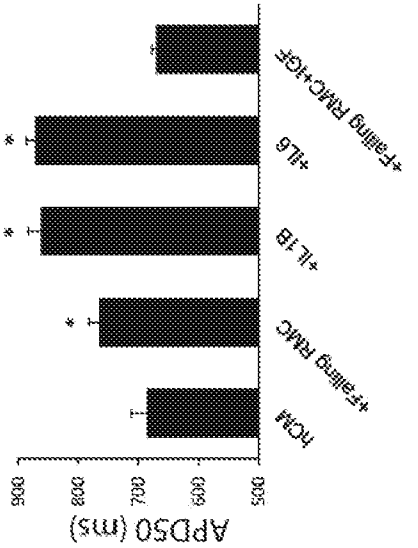

FIG. 9B

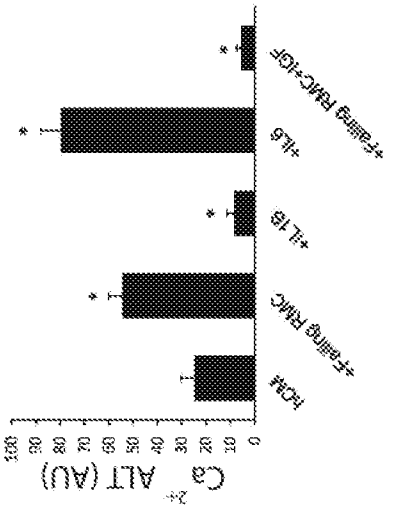

FIG. 9A

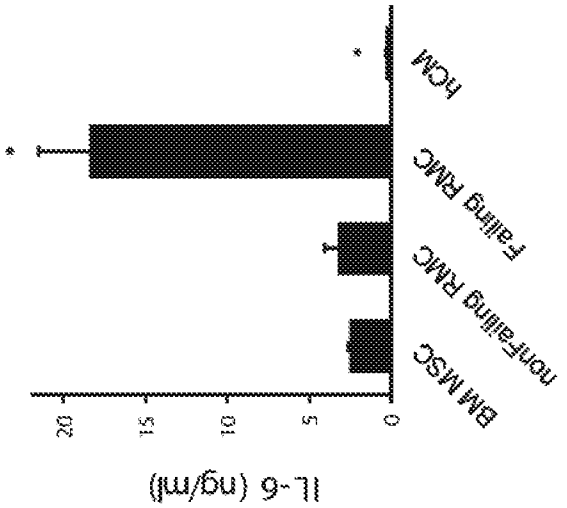
IL-6 (ng/ml)
0
5
10
15
20
BM MSC
nonFailing RMC
Failing RMC
hCM
*
*

FIG. 9E

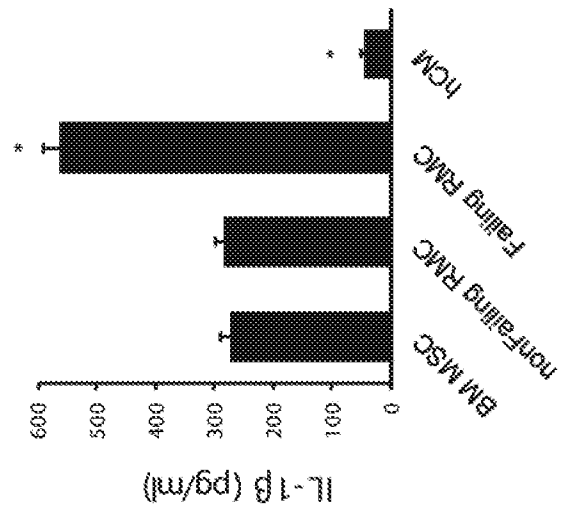
IL-1β (pg/ml)
0
100
200
300
400
500
600
BM MSC
nonFailing RMC
Failing RMC
hCM
*
*

FIG. 9D