Moderated Poster Session - Heart

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The involvement of TWEAK and FN14 in murine autoimmune myocarditis
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Background: Myocarditis, defined as an inflammation of the myocardium, can be caused by auto-
immune reactions. To study these immune reactions our group developed a mouse model of experi-
mental autoimmune myocarditis (EAM). Little is known about the role of the innate immune system in the pathogenesis of this autoimmune disorder. However, it was already observed that the cytokine tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and its receptor fibroblast growth factor-inducible 14 (FN14) play a pivotal role in different inflammatory diseases. Thus, we studied the involvement of TWEAK and FN14 in the development of inflammatory cardiomyopathy.

Methods and Results: AJ mice were inoculated with cardiac troponin I (cTnI) to induce an EAM followed by cardiomyogry, fibrosis and reduced ejection fraction (EF). To determine the severity of myocardial damage hsTnT levels were measured. Here, mice lacking FN14 (FN14−/−) displayed a signifi-

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Sympathetic neurons that innervate the heart locally modulate cardiomyocyte trophic and electrophysiological properties
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Purpose: The myocardium is highly innervated by Sympathetic Neurons (SNs), that distribute within the tissue with a well-defined pattern. We have previously shown that neuronal input to cardiomyo-
cytes (CMs) represses proteolysis and activates protein synthesis, through the β2-AR dependent sig-

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Glucocorticoid intervention prenatally: effects on fetal heart maturation
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Glucocorticoids are routinely administered to pregnant women at risk of pre-term delivery to mature fetal organs and improve neonatal survival. We have shown that glucocorticoid action is essential to

Characterized by different SN distribution, CM size follows neuronal density. Moreover, using cardiac optogenetics in denerated hearts, we observed changes in the transmural electrophysiology and in-
creased arrhythmia vulnerability, suggesting that in addition to controlling structural CM properties, the physiologic innervation pattern is fundamental to allow normal cardiac electrophysiology.

Conclusion: This is the first evidence, to the best of our knowledge, of an otherwise homogeneous tissue, shaped, after development, by a superimposed innervation network, through the modulation of cellular protein turnover.

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mature the fetal heart. Here, we tested the hypotheses that (i) antenatal glucocorticoid exposure, prior to the normal increase in glucocorticoid levels, will advance fetal heart maturation and (ii) this will depend on cardiovascular glucocorticoid receptor (GR).

Male SMGRKO mice, which have Sm22a-Cre mediated deletion of Gr in cardiomyocytes and vascular smooth muscle cells (VSMC), were crossed with female control (Cre-) mice to generate SMGRKO and control fetuses within the same pregnancy. Dexamethasone (Dex, 10μg/kg) or Vehicle (Veh) was administered in the drinking water of pregnant dams from E11.5 (n=3-6 dams/group), 2d prior to the initiation of fetal glucocorticoid synthesis. In utero high frequency ultrasound was performed at E15.5.

Initial results show that dexamethasone increased isovolumetric contraction time (indicating impaired myocardial contractility), was no significant effect in SMGRKO fetuses (Dex, 36.3 ± 6.1ms vs Veh, 25.1 ± 4.6ms; p < 0.05). However, there was no significant effect in SMGRKO fetuses (Dex, 31.4 ± 10.4ms vs Veh, 28.0 ± 9.3ms; p = 0.2). Heart rate was also decreased by Dex treatment in control (Dex, 191 ± 20bmp vs Veh, 227 ± 15 bmp; p < 0.05), but not in SMGRKO mice (Dex, 214 ± 7bmp vs Veh, 221 ± 7bmp; p = 0.5). Comparison of Veh treated SMGRKO and control mice showed no differences in E/A wave ratio, a marker of cardiac maturity (SMGRKO + Veh, 0.32 ± 0.03 vs Con + Veh, 0.43 ± 0.06; p = 0.49). However, in Dex treated mice, E/A wave ratio was lower in SMGRKO mice than in controls (SMGRKO + Dex, 0.32 ± 0.01 vs Con + Dex, 0.41 ± 0.01; p < 0.01), suggesting greater cardiac immaturity in SMGRKO mice than controls following exogenous glucocorticoid treatment. This finding is consistent with previous findings that glucocorticoid effects on E/A wave ratio are independent of cardiovascular GR.

Precocious glucocorticoid exposure may impair fetal septal heart function via activation of cardiovascular GR. It may also impair cardiac maturation via actions that are independent of cardiovascular GR. These could be mediated via GR elsewhere, via MR activation by endogenous corticosterone (in face of removal of competing GR) or via maternal or blood pressure effects. However, this requires further investigation and experiments are ongoing. Nevertheless, these data suggest that early exposure to potent synthetic glucocorticoids antenatally, prior to the normal increase in endogenous glucocorticoids, may be detrimental to cardiovascular health.

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Uncoupling of myofilament Ca2+ sensitivity from troponin I phosphorylation by hypertrophic and dilated cardiomyopathy mutations can be reversed by EGCG and related Hop90 inhibitors.

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Introduction: Heart muscle contraction is regulated by Ca2+ and modulated via the β-adrenergic response that leads to phosphorylation of Troponin I (TnI) at Ser 22/23, which changes the Ca2+ sensitivity of the cardiac myofilament. Previously, it has been shown that mutations in sarcomeric proteins that cause Dilated Cardiomyopathy (DCM) or Hypertrophic Cardiomyopathy (HCM) abolish the relationship between TnI phosphorylation and Ca2+ sensitivity. We have termed this “uncoupling”. Ca2+-desensitisers (such as Epigallocatechin-3-gallate (EGCG) and Silybin) act upon troponin and alter the Ca2+ sensitivity of the myofilament but their relationship with TnI phosphorylation has never been studied before. Using the in vitro motility assay (IVMA) we have found that Ca2+-desensitisers can reverse or “recouple” this relationship.

Purpose: To test the ability of EGCG and related compounds to reverse the uncoupling effect of DCM and HCM mutations.

Methods: The in vitro motility assay was mainly used in this study.

Results: EGCG decreased Ca2+ sensitivity of phosphorylated and unphosphorylated wild-type thin filaments equally retaining the coupling. In contrast, in thin filaments with the DCM-causing TPM1 E60K mutation, Ca2+-sensitivity was uncoupled. EGCG reduced Ca2+ sensitivity of phosphorylated but not unphosphorylated thin filaments containing DCM or HCM-causing mutations, reversing the uncoupling effect of the mutation. Thus, in the presence of EGCG, Ca2+ sensitivity dependence on phosphorylation is restored (re-coupling). The same pattern was observed with four other DCM mutations (TPM1 E54K, TNNC1 G159D, TNNT3 Q510N, ACTC E361G).

For the HCM-related TTN2 K280N mutation, troponin from a patient with this mutation showed no difference in Ca2+ sensitivity when compared with donor heart troponin and the Ca2+-sensitivity was also uncoupled. EGCG was able to restore coupling to this mutation and to other mutations in TTN2 (T14, 128, 73, R92Q, E160, S179F and K273E), TPM1 (E180G) and ACTC (E99K).

However, EGCG is promiscuous and has many off target effects and so an alternative compound is needed. 30 EGCG and Silybin related drug compounds were studied using IVMA for their ability to recouple. Some of these analogues indicated potent recoupling properties and their effects were preserved across three mutations causing DCM or HCM. As both EGCG and Silybin inhibit heat shock protein 90 (Hop90), preliminary investigations were made into 4 other Hop90 inhibitors (Resveratrol, Geldanamycin, Nobelobin and Pterostilbene), they were all found to be recoupers. The Ca2+-desensitising and recoupling effects of EGCG were also seen in contracting myofibrils.

Conclusion: The effect of EGCG and its analogues demonstrates that it is possible to reverse the pathological defects in troponin caused by DCM and HCM mutations pharmacologically and has the potential for treatment.

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Investigating inherited HCM caused by SCO2 and PRKAG2 mutations using the patients’ induced pluripotent stem cell-derived cardiomyocytes.

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Mutations in PRKAG2 gene encoding the γ subunit of AMPK cause hypertrophic cardiomyopathy (HCM) and familial Wolff-Parkinson-White syndrome (WPWv). Patients with R302Q mutation in PRKAG2 suffer from sinus bradycardia, escape rhythms, atrial fibrillation and supraventricular tachycardia. This mutation affects AMPK activity and causes elevated glycogen storage in cardiomyocytes. The link between glycogen storage and WPWv syndrome, HCM and arrhythmia remains unknown. A mutation in SCO2 gene encoding for mammalian cyclotron-α oxidase, a crucial part of the mitochondrial electron transport-chain, causes HCM and infants deaths. To investigate the pathological mechanisms underlying these HCM-causing mutations and search for novel pharmacological and genetic therapeutic modalities, we generated induced Pluripotent Stem Cells-derived cardiomyocytes (iPSC-CMs) from patients’ somatic cells, attempting to recapitulate the disease phenotype in vitro. The diseases we explored are: (1) HCM with familial WPWv caused by R302Q mutation in PRKAG2 gene. (2) Concentric HCM caused by G192S mutation in SCO2 gene. Successful reprogramming of respective patients’ skin-derived fibroblast resulted in iPSCs colonies expressing R302Q or G192S mutations. Action potentials were recorded from cardiomyocytes and extracellular electrograms from beating cardiomyocytes clusters using patch clamp and Micro Electrode Array (MEA) techniques, respectively. [Ca2+]i transients and contractions were recorded by means of a far-2 and video edge detector, respectively. The major findings were: (1) PRKAG2 mutated iPSC-CMs exhibited spontaneous delayed afterdepolarizations (DADs), slow firing rates and irregular rhythms (the latter two at the single cell and network level). Further, these phenomena were intensified with culture age, suggesting inter-relations between glycogen storage and electrophysiological abnormalities. (2) SCO2 mutated iPSC-CMs exhibited attenuated inotropic response to isoproterenol as well as DADs and irregular rhythms. Importantly, transmission electron microscopy analysis of SCO2-mutated iPSC-CMs displayed abnormal mitochondria size and morphology. Conclusion: PRKAG2 and SCO2 mutated iPSC-CMs displayed abnormal functional features resembling the clinical phenotype expressed in patients carrying the mutations. In these cases of life threatening arrhythmias the cause is neither mutations in structural proteins nor ion channels; the cause for arrhythmias involved with hypertrophic cardiomyopathy, here, lies within mutated metabolism regulators.