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EFFECTS OF A TAK-1 INHIBITOR AS A SINGLE OR AS AN ADD-ON THERAPY TO RIOCIGUAT IN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS FROM A RAT MODEL OF SEVERE PULMONARY ARTERIAL HYPERTENSION

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Introduction: Pulmonary arterial hypertension (PAH) is a complex disease involving vasoconstriction, thrombosis, inflammation, metabolic dysregulation and vascular proliferation, however all the drugs approved for PAH mainly act as vasodilating agents.
Objectives: Since excessive TGF-β signalling is believed to be a critical factor in pulmonary vascular remodelling, we evaluated the effects of blocking TGFβ-activated kinase 1 (TAK-1), alone or in combination with a vasodilator therapy in primary Pulmonary Arterial Smooth Muscle Cells (PASMC) isolated from a SU5416/hypoxia rat model of severe pulmonary arterial hypertension.
Methods: PAH was induced in male Wistar rats by a single injection of the VEGF receptor antagonist SU5416 (20 mg/kg) followed by exposure to hypoxia (10% O_2) for 21 days. At the end of the procedure, PASMC were isolated from Pulmonary Artery (PA) and seeded at 30000 cell/mL. Cells were growth arrested by an exposure for 24 h in 0.1% fetal calf serum (FCS) medium and then exposed to DMSO, (5z)-7-oxozeaenol, riociguat or both drugs combined. After 48 h, cells were fixed with 10 mL each of ice-cold methanol, chloroform, and deionized water. Cell extracts were analyzed using a 500 MHz Bruker spectrometer.
Results: Riociguat showed a negligible antiproliferative effects, while (5z)-7-oxozeaenol effectively inhibited proliferation of PASMCs. Furthermore, PASMC exposed to (5z)-7-oxozeaenol significantly attenuated the metabolic shift induced by SU5416/hypoxia protocol. Specifically, alanine, aspartate, creatine, creatinine, glutamate, glutamine, glycine, inosine monophosphate, 2-methylglutarate, isoleucine, lactate, phosphocholine, threonine and valine metabolic pools were found significantly decreased after the exposition to (5z)-7-oxozeaenol or the combination of (5z)-7-oxozeaenol and riociguat. In addition, combined treatment also induced a decrease in myo-inositol and proline concentrations. By contrast, the exposition to riociguat induced a decrease in lactate concentration.
Conclusions: Inhibition of TAK-1 induces antiproliferative effects and an effective attenuation of the metabolic alterations induced by PAH development.

HUMAN INFLUENZA A VIRUS CAUSES HEART INFECTION, CARDIAC CONDUCTION DISORDERS AND PREMATURE DEATH

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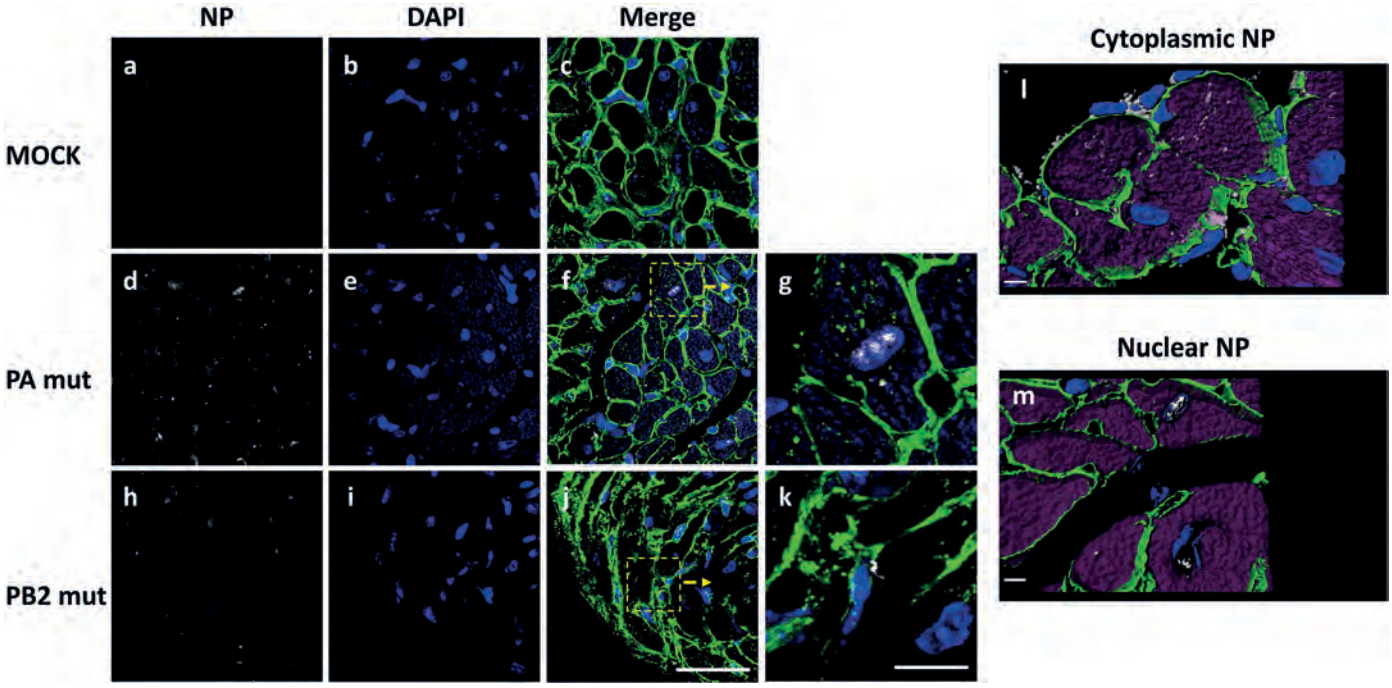
Introduction: Influenza A virus (IAV) infection is associated with important cardiovascular complications. However, current understanding considers cardiac IAV pathophysiology secondary to respiratory damage.
Objectives: To study the ability of IAV of different pathogenicity to infect the hearts of mice, and establish the potential relationship between the infective capacity in heart tissue and cardiac alterations.
Methods: We evaluated lung and heart viral titers in mice infected with either one of several human viral strains of influenza virus inoculated intranasally. We evaluated the presence of viral proteins inside cardiomyocytes and Purkinje cells by 3D reconstructions of infected cardiac tissue. We also measured viral replication in mouse cultured cardiomyocytes, human iPSCs-derived cardiomyocites (hiPSC-CMs), and conducted sequential electrocardiograms and cardiac magnetic resonance imaging to evaluate functional and structural consequences.
Results: Pathogenic and attenuated human IAV strains infected cardiomyocytes and Purkinje cells. High viral titers in the lungs of infected mice did not necessarily coincide with high viral titers in the heart. The highly pathogenic recombinant virus PAmut, bearing a mutation (D529N) responsible for the augmented pathogenicity of a human fatal-case IAV, could replicate faster and showed a higher final viral titer in cardiac HL-1 mouse cells than the attenuated virus PB2mut. Correspondingly, bradycardia, and electrical conduction alterations were especially pronounced in PAmut-infected mice, which also



Active replication of IAV in cardiomyocytes. Detection of viral nucleoprotein (NP) by confocal microscopy of immunofluorescences in heart tissue from control animals (MOCK; upper panels, a-c) or animals infected with 106 pfu of either PAmut virus (middle panels, d-f) or PB2mut virus (lower panels, h-j). Scale bar, 40 µm. The boxed areas in merged images (f and j) are shown enlarged in the insets (g, k). Scale bar for insets: 10 µm. Right panels (l-m), 3D reconstructions of cytoplasmic or nuclear NP viral protein in PAmut IAV infected heart tissue. Blue, nucleus staining (DAPI); green, laminin; magenta, auto-fluorescence in cardiomyocytes; white, NP viral protein staining. Scale bar, 5 µm.

showed higher mortality rates compared to PB2mut. *Ex-vivo* post-contrast T1 mapping sequences did not show significant cardiac structural differences among infected groups. Molecular alterations were detected in infected hiPSC-CMs which may explain some of the alterations observed *in vivo* in infected mice.
Conclusions: Influenza virus can infect the heart and cardiac specific conduction system, which may contribute to cardiac complications and premature death.

IMPACT OF VITAMIN D DEFICIT AND PULMONARY HYPERTENSION ON THE RAT GUT MICROBIOME

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Inadequate immunologic, metabolic and cardiovascular homeostasis has been related to either an alteration of the gut microbiota or to vitamin D deficiency. We analyzed whether vitamin D deficiency alters rat gut microbiota. Male Wistar rats were fed a standard or a vitamin D-free diet for seven weeks. The microbiome composition was determined in fecal samples by 16S rRNA gene sequencing. The vitamin D-free diet produced mild changes on α-diversity but no effect on β-diversity in the global microbiome. Markers of gut dysbiosis like *Firmicutes*-to-*Bacteroidetes* ratio or the short chain fatty acid producing bacterial genera were not significantly affected by vitamin D deficiency. Notably, there was an increase in the relative abundance of the *Enterobacteriaceae*, with significant rises in its associated genera *Escherichia*, *Candidatus blochmannia* and *Enterobacter* in vitamin D deficient rats. *Prevotella* and *Actinomyces* were also increased and *Odoribacteraceae* and its genus *Butyricimonas* were decreased in rats with vitamin D-free diet. In conclusion, vitamin D deficit does not induce gut dysbiosis but produces some specific changes in bacterial taxa, which may play a pathophysiological role in the immunologic dysregulation associated with this hypovitaminosis.

NEW INSIGHTS INTO THE ECMO SUPPORT WITHIN THE UK MODEL

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Introduction: Extracorporeal supports have experimented a huge expansion during last decades. Given the technological development and lack of evidence-based consensus for clinical management, generating scientific knowledge in this field is mandatory for the critical care community. Fellowships at high-volume ECMO intensive care units may be an opportunity to acquire the detailed know-how surrounding these techniques and foster new collaborative projects.
Objectives: To gain experience on clinical practice, join and participate with on-going clinical studies and promote new collaborative research lines, at an external reference ECMO-centre.
Methods: The selected institution was the Guy's and St Thomas' NHS Foundation Trust, one of the 5 centres in the UK commissioned to offer ECMO for respiratory failure in adults. This unit is also involved in relevant clinical trials (PROTECMO, REST). Thanks to the funding obtained from CIBERES, a three-month stay was possible and resulted in an enriching experience.
Results: The applicant participated in a recently published study aimed to describe the pattern of CT-recruitability in severe ARDS and its relationship with defined outcomes. Also, the role of carbon dioxide gaps as predictors of low cardiac output was explored. A nomogram to calculate the blood CO_2 content at the bedside was elaborated. This model was then applied to a dataset of 6,000 pairs of gases to validate its usefulness for discriminating patients at high risk of inadequate cardiac output (unpublished results). Additionally, the applicant pioneered an interventional clinical study back at her institution, in collaboration with the local team. The objective was to evaluate the

feasibility and safety of ultraprotective ventilation in patients with cardiogenic shock and va-ECMO. Lung inflammatory response to different ventilatory settings was compared in this context.
Conclusions: Institutional support through funding opportunities favours the interaction and synergies with strategic external research groups and is key for increasing its own scientific production and internationalization.

ROLE OF KV7 CHANNELS AND KCNE ANCILLARY SUBUNITS IN THE PULMONARY VASCULATURE: POSSIBLE IMPLICATION IN PULMONARY HYPERTENSION

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Introduction: Pulmonary arterial hypertension (PAH) is characterised by vasoconstriction, thrombosis and vascular remodelling in pulmonary arteries (PA). K+ channels play a fundamental role regulating membrane potential (Em) of smooth muscle cells (PASMCs). Recently, a key role of Kv7 channels in the control of vascular tone has been demonstrated. Moreover, reduced Kv7 channel activity has been observed in different cardiovascular pathologies such as diabetes, hypertension or long QT syndrome.
Objectives: To study the role of Kv7 channels/KCNE subunits in the pulmonary vasculature and their possible alteration in PAH.
Methods: Kv7 channel activity was analyzed using the whole-cell configuration of the patch-clamp technique and vascular reactivity in a PAH-animal model. The expression of Kv7 channels/KCNE subunits was analyzed by qRT-PCR and Western blot in lungs from PAH model. Cell localization studies were analyzed using immunocytochemistry and proximity ligation assay.
Results: The data showed a decrease in K+ current and a more depolarized Em in PAH-PASMCs compared to controls. Despite, the contribution of Kv7 channels was higher in PAH-PASMCs than controls. The vascular reactivity study also showed an enhanced response to Kv7 channels modulators in PAH-PA compared with control-PA where, the Kv7 channels enhancers produce a negligible relaxation. These data were supported by the cellular localization study, which showed that Kv7 channels/KCNEs subunits expression in control-PA was essentially cytosolic. Interestingly, we found an altered expression of Kv7 channels/KCNE subunits in PAH-lungs: a decrease in Kv7.4α and an increase in KCNE4 subunit, which is consider an enhancer subunit of Kv7 channels and is involved in the trafficking of these channels to the membrane.
Conclusions: In conclusion, control-PA present a reduce Kv7 channel function since the expression is cytosolic in physiological conditions. However, in PAH there is a gain-of-function of Kv7 channels, which could be mediated by the KCNE4 up-regulation and could suppose a possible therapeutic target in PAH.

TRANSCRIPTOMICAL ANALYSIS OF THE HUMAN TUBERCULOSIS LESIONS THROUGH NEXT GENERATION SEQUENCING

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Introduction: Tuberculosis (TB) is a chronic infectious disease that affects globally more than 10 million people per year. Nowadays there is no validated marker for protection, diagnosis nor prognosis. The goal of this study is to assess the gene expression in TB patients that underwent therapeutic surgery, examining removed lung tuberculosis granuloma samples and host's blood through transcriptomic methods. We expect that the correlation of the results with patient's clinical and microbiological traits may allow stablishing a specific biomarker gene panel to be useful in patient's management.
Objectives: 1. To analyze the obtained total-RNA from samples through Next Generation Sequencing (NGS). 2. To perform the differential expression analysis between different granuloma parts and against the healthy lung tissue.
Methods: Clinical and microbiological data was collected from 14 TB patients that underwent therapeutic surgery in Tbilisi, Georgia. Total-RNA was obtained from 13 pre-surgery blood tubes, 32 granuloma and 13 healthy lung tissue samples following cryofracturing and grinding methods. After NGS, raw-data reads were aligned against the human reference genome to obtain the counts through STAR. DESeq2 allowed to perform the differential expression analysis.
Results: A TB granuloma gene signature was obtained from the three granuloma zones under analysis. A total of 1842 genes are significant differentially expressed between the TB granuloma tissue and healthy lung parenchyma. The spatial analysis revealed that granuloma inner-parts gather unique and dysregulated genes from healthy tissue.
Conclusions: 1. The healthy lung parenchyma have a different total-RNA expression pattern from granuloma's signature. 2. The human TB granuloma is spatial-transcriptomically organized. 3. The granuloma signature comparison with other TB public datasets is ongoing.