TRANSLATIONAL MEDICAL RESEARCH OF CARDIOVASCULAR DISEASE

GW28-e0098

Interleukin-35 is a Potent Inhibitory Cytokine that Regulates Dendritic Cell Maturation and Subsequently Increases Regulatory T Cells

Xianglan Liu,[1,2] Yong Sun,[1,2] Bo Yu[1,2]
[1]The Key Laboratory of Myocardial Ischemia, Harbin Medical University, Ministry of Education, Heilongjiang Province, China; [2]Department of Cardiology, the Second Affiliated Hospital of Harbin Medical University, Harbin, China

OBJECTIVES Interleukin-35 (IL-35) was identified as a novel anti-inflammatory cytokine composed of IL-12p35 (p35) and Ebi3. We hypothesized that IL-35 is a potent inhibitory cytokine that regulates dendritic cell (DC) maturation and subsequently increases regulatory T cells.

METHODS Bone marrow-derived DCs were cultured for 7 days in the presence of IL-4 and GM-CSF to obtain immature DCs and then treated with LPS, TNF-α, IFN-γ or IL-1 for 24h. An ELISA assay kit was used to detect the expression levels of IL-12 cytokine family. The expression levels of IL-12p35 and Ebi3 mRNA were detected by qRT-PCR in DCs. DCs were treated with exogenous IL-35/Ebi3 or Ebi-3 neutralizing antibody for 24h before stimulation with LPS. The mature DC subtypes (CD80,CD86,MHC-II) were analyzed using flow cytometry. DCs were harvested and co-cultured with splenocytes. The T cells were incubated with $CD4^+$, $CD25^+$, and $Foxp3^+$ antibodies or $CD4^+$ and $IL\text{-}17A^+$ antibodies. T cells were detected using flow cytometry. STAT activation in differently treated DCs was analyzed by Western blotting. DCs were transfected with a miR-let-7i mimic or an inhibitor or si-SOCS1 (60 nM), the expression levels of SOCS1 and IL-12 family were then detected.

RESULTS Here, we showed that IL-35 expression was significantly increased in a time-dependent manner in mouse mature dendritic cells (mDCs) stimulated by lipopolysaccharide (LPS) but not by TNF-α, IFN-γ and IL-1.Recombinant IL-35 suppressed DC maturation after stimulation by LPS and increased the secretion of anti-inflammatory cytokines. Co-culturing IL-35-treated DCs with T cells significantly increased the population of $CD4^+CD25^+Foxp3^+$ T cells. IL-35 may activate STAT1, STAT3 and STAT4 in DCs to achieve its biological function.MicroRNA let-7i (miR-let-7i), as the prototype of the miR-let-7 family, could inhibit IL-35 expression by targeting suppressor of cytokine signaling 1 (SOCS1).

CONCLUSIONS IL-35 expression was significantly increased in LPS-induced DC maturation.Exogenous IL-35 can regulate the maturation and function of DCs.MiR-let-7i regulates IL-35 secretion in DCs by targeting SOCS1.Collectively, our data show that IL-35-treated DCs may be a promising approach to regulate immunity.

GW28-e0175

In silico assessment of the effects of drug disopyramide on electrical activity in human ventricular myocardium associated with short QT syndrome

Cunjin Luo,[1] Kuanquan Wang,[1] Henggui Zhang[1,2,3]
[1]School of Computer Science and Technology, Harbin Institute of Technology (HIT), Harbin, China; [2]School of Physics and Astronomy, The University of Manchester, Manchester, M13 9PL, United Kingdom; [3]Space Institute of Southern China, Shenzhen, China

OBJECTIVES Short QT syndrome (SQTS) is an inheritable electric heart disease characterized by abnormally short QT intervals on the ECG and a high incidence of sudden cardiac death (SCD) in individuals with a structurally normal heart. To our knowledge, multiple functional effects of arrhythmogenic mutations in SQTS have been studied, but relatively little is known about the potential pharmacological treatment for SQTS patients. Therefore, in this study, we aimed to simulate the Class Ia anti-arrhythmic drug disopyramide on electric activity in human ventricular myocytes associated with SQT1, SQT2, and SQT3 SQTS variants.

METHODS Short QT syndrome (SQTS) is an inheritable electric heart disease characterized by abnormally short QT intervals on the ECG and a high incidence of sudden cardiac death (SCD) in individuals with a structurally normal heart. To our knowledge, multiple functional effects of arrhythmogenic mutations in SQTS have been studied, but relatively little is known about the potential pharmacological treatment for SQTS patients. Therefore, in this study, we aimed to simulate the Class Ia anti-arrhythmic drug disopyramide on electric activity in human ventricular myocytes associated with SQT1, SQT2, and SQT3 SQTS variants.

RESULTS At a clinically-relevant therapeutic concentration of 10 μM disopyramide (the fractional block on affected ionic currents: under SQT1 condition: I_{Kr}: 42.9%, I_{Na}: 4.4%, I_{to}: 3.0%, and I_{CaL}: 1.0%; under SQT2 and SQT3 conditions: I_{Kr}: 48.3%, I_{Na}: 4.4%, I_{to}: 3.0% -ECG was prolonged in SQT1 (from 286 ms in SQT1 N588K condition to 340 ms in the presence of disopyramide), but with insignificant effect in SQT2 (from 338 ms in SQT2 heterozygous condition to 348 ms in the presence of disopyramide) and SQT3 (from 322 ms in SQT2 heterozygous condition to 336 ms in the presence of disopyramide). Moreover, T-wave amplitude was markedly decreased in SQT1 with the application of disopyramide.

CONCLUSIONS Our simulation data show that disopyramide has a markedly prolonged QT interval effect on SQT1. This study helps to better understand the underlying mechanisms of pharmacological therapy, and provides further evidence that disopyramide may be a suitable treatment for SQT1, rather than SQT2 and SQT3.

GW28-e0180

Bay60-2770 attenuates doxorubicin cardiotoxicity by prevention of mitochondria membrane potential loss

Xiaoxiao Zhao,[1,2] Guan Like[1]
[1]Yanbian University Hospital, China; [2]Kyunghee University Hospital, Korea

OBJECTIVES Previous studies have attributed the cause of doxorubicin (DOX) mediated cardiotoxicity to mitochondrial iron accumulation and the ensuing reactive oxygen species (ROS) formation. The present study investigates the role of Bay60-2770, a more effective activator of oxidized soluble guanylate cyclase (sGC), and its role in alleviating DOX mediated cardiotoxicity.

METHODS H9c2 cardiomyocytes were pre-treated with BAY60-2770 followed by DOX, and cell viability and intracellular reactive oxygen species (ROS) were subsequently measured. In order to determine the role BAY60-2770 in mitochondrial ROS generation and mitochondrial membrane potential, we examined MitoSOX RED and TMRE fluorescence under DOX exposure. Additionally, rats were orally administered with BAY60-2770 1 hour prior to every DOX treatment. Left ventricular (LV) function and performance were then assessed by echocardiography. Mitochondrial iron regulating protein expression levels were examined by western blot analysis.

RESULTS BAY60-2770 ameliorated cell viability and oxidative stress induced by DOX in H9c2 cardiac myocyte, which mediated by PKG activation. Mitochondrial ROS and TMRE fluorescence attenuated by BAY60-2270 in DOX-treated H9c2 cells. DOX-induced caspase-3 activation decreased after pre-treatment with BAY60-2770 both *in vivo* and *in vitro*. Echocardiography showed that pre-treatment with BAY60-2770 significantly improved reduced LV function that is induced by DOX treatment. BAY60-2270 enhanced the protein expression of Mitochondrial ferritin (MtFt) in DOX administered heart.

CONCLUSIONS BAY60-2770 reduces DOX-induced mitochondrial membrane potential loss and subsequent apoptosis by up-regulating MtFt and improves cardiac function. These novel results highlight the therapeutic potential of BAY60-2770 to prevent doxorubicin cardiotoxicity.

GW28-e0387

Targeting amino acid metabolism for molecular imaging of inflammation early after myocardial infarction

Jie Qin,[1] Chuangfeng Li,[1] Lingrong Peng,[1] Wenjie Tang[1]
[1]Department of Radiology, the Third Affiliated Hospital of Sun Yat-sen University

OBJECTIVES To assess the suitability of positron emission tomography (PET) with (11)C-methionine for imaging post- myocardial Infarction (MI) inflammation from cell to mouse to man.

METHODS Uptake assays demonstrated 7-fold higher (11)C-methionine uptake by polarized pro-inflammatory M1 macrophages over anti-inflammatory M2 subtypes ($p<0.001$). C57Bl/6 mice ($n=27$) underwent coronary artery ligation or no surgery. Serial (11)C-methionine PET was performed 3, 5 and 7d later.

RESULTS MI mice exhibited a perfusion defect in 32-50% of the left ventricle (LV). PET detected increased (11)C-methionine accumulation in the infarct territory at 3d (5.9±0.9%ID/g vs 4.7±0.9 in remote myocardium, and 2.6±0.5 in healthy mice; $p<0.05$ and <0.01 respectively), which declined by d7 post-MI (4.3±0.6 in infarct, 3.4±0.8 in remote; $p=0.03$ vs 3d, $p=0.08$ vs healthy). Increased (11)C-methionine uptake was associated with macrophage infiltration of damaged myocardium. Treatment with anti-integrin antibodies (anti-CD11a, -CD11b, -CD49d; 100μg) lowered macrophage content by 56% and (11)C-methionine uptake by 46% at 3d post-MI. A patient study at 3d after ST-elevation MI and early reperfusion confirmed elevated (11)C-methionine uptake in the hypoperfused myocardial region. Targeting of elevated amino acid metabolism in pro-inflammatory M1 macrophages enables PET imaging-derived demarcation of tissue inflammation after MI.

CONCLUSIONS (11)C-methionine-based molecular imaging may assist in the translation of novel image-guided, inflammation-targeted regenerative therapies.

GW28-e0552

Impact of timing and repeated doses of transplantation of atorvastatin-pretreated mesenchymal stem cells for treating acute myocardial infarction

Jun Xu,[1] Yuejin Yang[1]
[1]State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College

OBJECTIVES Mesenchymal stem cells (MSCs), as one of the main types of stem cells, are widely utilized in cardiac repair. However, due to their low engraftment rate within the ischemic myocardium, MSCs therapy has left much to be explored. Our previous studies found that the combination of loading dose of atorvastatin (ATV) and single injection of ATV-pretreated MSCs at 1 week after acute myocardial infarction (AMI) can promote the engraftment of MSCs and enhance the cardiac performance in AMI rats to a degree. The aim of this study was to investigate whether the combination of loading dose of ATV and repeated ATV-pretreated MSCs injections in different stages of AMI have greater efficacy than single injection.

METHODS 180 female Sprague-Dawley rats were randomized into 11 groups, including Sham, AMI control; Early-1 (single injection in early stage at day 3), Early-2 (dual injections in early stage at day 3 and day 7), Early-3 (triple injections in early stage at day 1, day 3 and day 7); Mid-1 (single injection in mid-term stage at day 7), Mid-2 (dual injections in mid-term stage at day 7 and day 11), Mid-3 (triple injections in mid-term stage at day 7, day 11 and day 14); Late-1 (single injection in late stage at day 21), Late-2 (dual injections in late stage at day 21 and day 25), and Late-3 (triple injections in late stage at day 21, day 25 and day 28). Myocardial infarction was created by left anterior descending (LAD)ligation. All groups except Sham and AMI control were giving loading dose of ATV every day. ATV-pretreated MSCs labeled with CM-Dil were intravenous injected and the time point and times of infusion were listed in the parentheses above. At 5th week after AMI, cardiac functions were assessed using echocardiography and left heart catheter pressure measurement. Recruitment of MSCs to the infarcted heart, inflammatory cells and fibrosis were evaluated with histopathology.

RESULTS Compared to AMI control group, cardiac performance was improved in all nine experiment groups with an increased left ventricular ejection fraction (LVEF) and decreased infarct area. In the early and mid-term stage, both LVEF and infract area of groups undergoing multiple injections of ATV-pretreated MSCs were more favorable than that of single injection, which demonstrated a cumulative effect. There were, however, no significant differences between the either two groups in the late stage. The optimal treatment group was Mid-3, with highest LVEF (baseline LVEF: 50.2±17.9%; endpoint LVEF: 65.9±10.0%) at the endpoint. The infarct area of Mid-3 group was just one third of AMI control group. In contrast, among the nine experiment groups, Early-1 group had the least improvement both in LVEF and infarct area.

CONCLUSIONS On the basis of loading dose of ATV, repeated injections of ATV-pretreated MSCs did demonstrate better efficacy than single injection. And the optimal timing of repeated stem cell transplantation was from 7 days to 14 days after myocardial infarction. This study has provided preclinical evidence for repeated stem cell transplantation therapy of acute myocardial infarction.

GW28-e0652

Meta-analysis of Bone Marrow Stem Cell Therapy on Patients with Chronic Ischemic Heart Disease and Congestive Heart Failure

Zhe Xu,[1] Jin Fan,[2] Xi Zhang[1,3]
[1]Division of Cardiac Surgery, Department of Cardiovascular Diseases, the First Affiliated Hospital of Sun-Yat-sen University; [2]Division of Obstetrics and Gynecology, the First Affiliated Hospital of Jinan University; [3]Division of Laboratory of Respiratory, Department of Respiratory Diseases, the First Affiliated Hospital of Guangzhou Medical University

OBJECTIVES Bone marrow stem cell therapy as an option for regenerative therapy in chronic ischemic heart disease (IHD) and congestive heart failure was tested in a few randomized controlled trials (RCTs) but with no consistent conclusions regarding salubrious effects in enhancing heart function, remodeling and improving outcomes. Our aim was to provide a pooled estimate of safety and potential benefit of stem cell therapy on these patients.

METHODS Relevant RCTs published before Jan. 2017 were collected in a number of databases and analyzed with RevMan 5.3. Primary outcomes were all-cause mortality and left ventricular ejection fraction (LVEF) in the short-term follow-up (< 12 months) and long-term follow-up (more than or equal to 12 months). Secondary outcomes included left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), and stroke volume index.

RESULTS Twenty six RCTs with 1451 patients were included. Pooled analysis showed the bone marrow stem cells (BMSC) treatment had a trend toward lower risk of mortality in the short-term follow-up (risk ratio (RR) 0.59, 95% confidence interval (CI) 0.32 to 1.08, $P = 0.09$), and significantly reduced the incidence of mortality in the long-term follow-up (RR 0.28, 95% CI 0.15 to 0.55, $P = 0.0002$). The treatment was also associated with an improvement in LVEF both in the short-term (mean difference (MD) 4.17%, 95% CI 3.42% to 4.92%, $P< 0.00001$) and long-term follow-up (MD 2.62%, 95% CI 0.50% to 4.73%, $P= 0.015$). Moreover, the pooled results showed a significant difference in the stroke volume index(3.84, 95% CI: 0.95 to 6.73, $P= 0.0093$) and the mean LVESV (-5.47 ml, 95% CI: -8.81 ml to-2.14 ml, $P= 0.0013$), in favor of BMSC therapy in the short-term follow-up. The incidence of adverse event in BMSC-based therapies was generally low in most included studies. No significant difference was noted in other outcomes.

CONCLUSIONS Bone marrow stem cell therapies are generally safe and have a potential beneficial clinical effect on IHD patients with congestive heart failure in the short-term and long-term follow-up.

GW28-e0775

Bone marrow stem cells support human islet β-cell function in an encapsulated microenvironment

Feng Wu[1]
[1]shanghai changzheng hospital

OBJECTIVES Islets transplantation holds promise as a long term treatment to Type I diabetes. We have previously reported that bone marrow (BM) cells co-cultured with human islets generate a microenvironment suitable for repairing islets and promoting longevity. However, overcoming immunorejection is still a great challenge. We hypothesize that encapsulated islet/BM enhances human islet function while preventing immunorejection.

METHODS APA encapsulation was established by coating gel beads with additional layers of poly-L-ornithine and alginate to create a 4-layered immunoisolatory membrane. Fresh human islets were co-encapsulated with or without fresh human BM (1, 5 and 10 x 10^6) in APA microcapsules and the optimal ratio of BM to islets was studied based on insulin secretion using ELISA.

RESULTS No labeled peripheral blood cells (PBCs) were observed inside the capsule with human islets, indicating the APA encapsulation isolated human islets from PBCs to create a unique microenvironment free from potential interaction with host immune responses. Human islets (2500 IEQ) co-encapsulated with 5 x 10^6 BM generated the most optimal results over a 5 week culture period. After 4 weeks of culture, encapsulated human islets with BM formed a 3D structure while groups without encapsulation formed a 2D structure. Encapsulated human islets with 5 x 10^6 BM released more insulin when stimulated than groups without encapsulation under otherwise the same conditions.