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

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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.

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

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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.

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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.

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

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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.

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

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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.