Translational Medical Research of Cardiovascular Disease

GW25-e0820

Apoptosis of THP-1 macrophage-derived foam cells induced by 5-aminolevulinic acid-mediated sonodynamic therapy is mitochondria-caspase pathway predominant despite the participation of endoplasmic reticulum stress

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Objectives: In advanced atherosclerosis, chronic endoplasmic reticulum (ER) stress induces foam cells apoptosis and generates inflammatory reactions. Sonodynamic therapy (SDT) is a non-thermal synergistic method for cancer treatment utilizing low-intensity ultrasound and sonosensitizers. 5-Aminolaevulinic acid (ALA) is the biological precursor of sonosensitizer PpIX in the heme biosynthesis pathway in mitochondria. In this study, we investigated the sub-cellular location of ALA-PpIX in THP-1 macrophage-derived foam cells (FC) and the activation of mitochondria pathway and ER stress induced by ALA-SDT.
Methods: FC were incubated with 1 mM ALA. Fluorescence spectrometer was used to detect the location and metabolism of ALA-PpIX in mitochondria and endoplasmic reticulum (ER) of FC. Annexin V-PI staining was used to optimize ALA-SDT treatment parameters by detecting the apoptotic and necrotic rates of FC induced by ALA-SDT with different ALA incubation time and ultrasonic irradiation intensities. Intracellular reactive oxygen species (ROS) level after ALA-SDT was detected by staining with CellROX® Green Reagent. Mitochondrial membrane potential after ALA-SDT was detected by staining with JC-1. Pretreated with ROS inhibitor N-acetylcysteine (NAC), pan-caspase inhibitor Z-VAD-FMK and ER stress inhibitor 4-phenylbutyrate (4-PBA), expressions of mitochondria apoptosis associated proteins cytochromc c, cleaved caspase3, cleaved caspase9, Bcl-2, BAX and ER stress associated protein C/EBP-homologous protein (CHOP) in FC after ALA-SDT were detected by Western blotting.
Results: Accumulation of ALA-PpIX in mitochondria and ER reached peak at 6-hour, and the fluorescence intensity in mitochondria was triple of that in ER. The highest percentage of apoptotic cells (63.6%±9.8%) and the maximum apoptosis/necrosis ratio (21.7±6.3) was observed at 5-hour after ALA-SDT with 6-hour incubation of ALA and 0.4 W/cm^2 ultrasound intensity. After ALA-SDT, intracellular ROS level increased and the mitochondrial membrane potential collapsed. The translocations of cytochrome c from mitochondria into cytosol and Bax from cytosol into mitochondria, cleaved caspase 9, cleaved caspase 3, downregulation of Bcl-2, as well as upregulation of CHOP were detected at 5-hour after ALA-SDT, which could be suppressed by NAC. Activation of mitochondria apoptosis pathway could not be inhibited by 4-PBA. Apoptosis induced by ALA-SDT could be inhibited by Z-VAD-FMK. 4-PBA reduced FC apoposis by one third.
Conclusions: Mitochondria-caspase pathway is predominant in the apoptosis of FC induced by ALA-SDT though ER stress participates in.

GW25-e1534

SIRT1 protects against oxidative stress-induced endothelial progenitor cells apoptosis by inhibiting FOXO3a via FOXO3a ubiquitination and degradation

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Objectives: Endothelial progenitor cells (EPCs) -based therapy holds tremendous promise for the treatment of ischemic diseases. However, the function and survival of EPCs are dramatically impaired by oxidative stress. SIRT1 plays important roles in many pathophysiological processes such as apoptosis by deacetylation various substrates, including FOXO. However, little is known about the roles of SIRT1 in the regulation of EPCs apoptosis induced by H_2O_2. Our previous work showed that FOXO3a could promote apoptosis of EPCs by transcriptional regulation of Bim. In the present study, we investigated whether SIRT1 exerted a protective effect against H_2O_2-induced EPCs apoptosis and whether SIRT1 deacetylation of FOXO3a could facilitate FOXO3a ubiquitination and subsequent degradation.
Methods: EPCs were isolated and obtained from human umbilical cord blood by density gradient centrifugation. Incubation of EPCs with H_2O_2 was used to induce apoptosis. Apoptosis was determined by flow cytometry and DNA fragmentation. Western Blot analysis was used to examine the expression of SIRT1, FOXO3a, Bax and cleaved caspase 3. Adenoviral-mediated transfection was used to overexpress or downregulate SIRT1. Co-immunoprecipitation (co-IP) assay was performed to test the interaction between SIRT1 and FOXO3a, FOXO3a acetylation level and FOXO3a ubiquitination level.
Results: Immunofluorescence showed that SIRT1 localized in the nuclear of EPCs in the absence or presence of H_2O_2. SIRT1 expression in EPCs was increased by the treatment with H_2O_2 (500μM) for 24 hours. Incubation of EPCs with H_2O_2 dose dependently induced EPCs apoptosis. SIRT1 overexpression reduced H_2O_2-induced EPCs apoptosis, while SIRT1 downregulation and EX527, a specific SIRT1 inhibitor, exerted the opposite effect. SIRT1 overexpression decreased the total FOXO3a expression, whereas SIRT1 downregulation and EX527 increased the amount of FOXO3a. Co-IP assay showed that SIRT1 could bind to FOXO3a, reduce its acetylation level and increase its ubiquitination level.
Conclusions: The results of our work demonstrated that SIRT1 had a pivotal protective role in the regulation of EPCs apoptosis induced by H_2O_2 and that SIRT1 protected against apoptosis by inhibiting FOXO3a via FOXO3a ubiquitination and subsequent degradation.

GW25-e0540

Pharmacogenetics of clopidogrel responsiveness in Chinese patients with acute coronary syndrome

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Objectives: Cytochrome P450 (CYP), ATP-binding cassette transporters (ABCB1), and paraoxonase-1 (PON1) play crucial roles in clopidogel metabolism. Genetic polymorphisms in these genes have been associated with the variability of the response to clopidogrel, however, there are controversies over the findings. The objective of the study is to elucidate the contribution of genetic polymorphisms in CYP2C19, ABCB1, and PON1, to clopidogrel responsiveness in Chinese acute coronary syndrome (ACS) patients.
Methods: Five hundred Chinese-Han patients treated with clopidogrel for ACS were consecutively recruited. We assessed the relationships of CYP2C19*2, CYP2C19*3, CYP2C19*17, PON1Q129R and ABCB1C3435T to the on-treatment platelet reactivity (OTPR) after 5 days maintenance dose of clopidogrel administration, and the risk for high on-treatment platelet reactivity (HPR, defined as 20μmol/L ADP-induced platelet aggregation>50%). In addition, clopidogrel responsiveness, measured by RI [(pretreatment aggregation-posttreatment aggregation at 5 days) / (pretreatment aggregation) x100%], was assessed in relation to the genotypes in a subgroup of 180 patients. RI values were stratified into four quartiles, with patients in quartile 1 defined as individuals of clopidogrel non-responsiveness.
Results: Both CYP2C19*2 and *3 were significantly associated with higher OTPR ($P<10^{-5}$ and P=0.04, respectively). OTPR in carriers of at least one CYP2C19 loss-of-function allele (*2 or *3, accounted for 58% of the study population) was obviously higher than that in CYP2C19 wild type carriers ($P<10^{-5}$). The carriers of at least one CYP2C19 loss-of-function allele could predict greater risk of HPR (adjusted OR: 1.79, 95% CI: 1.33-2.4, P=0.003). Patients with CYP2C19*2 alone, instead of CYP2C19*3, had a higher risk for HPR (adjusted OR: 1.56; 95% CI 1.04-2.33, P=0.03). No significant relation of CYP2C19*17, PON1Q192R and ABCB1C3435T to OTPR and HPR was found in the cohort. In the subgroup of 180 patients, RI values were significantly lower in patients with PON1 192 QR and RR than in patients with QQ alleles (P=0.01). PON1 192 QR and RR conferred increased risk for clopidogrel non-responsiveness [adjusted OR: 3.64; 95% CI (1.21-10.92), P=0.02]. A trend for lower RI values was shown in CYP2C19*2 carriers compared to CYP2C19 wild type carriers (P=0.06). An increased risk for clopidogrel non-responsiveness was found in patients with CYP2C19*2 [adjusted OR 2.02; 95% CI (1.03-3.96), P=0.04]. No significant relation of CYP2C19*3, CYP2C19*17, and ABCB1C3435T to RI was found in the subgroup of patients.
Conclusions: In conclusion, in clopidogrel treated Chinese patients with ACS, carriers of at least one CYP2C19 loss-of-function allele could predict greater risk for HPR, with the impact mainly attributing to CYP2C19*2. Both PON1 Q192R and CYP2C19*2 genotypes influence clopidogrel responsiveness, with the impact of PON1 Q192R mainly on relative platelet inhibition instead of HPR of clopidogrel.

GW25-e0542

Effect of intracoronary or intravenous tirofiban bolus administration on platelet activity and short time clinic benefit in patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment

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Objectives: To investigate the effect on platelet activity and short time clinic benefit of intracoronary or intravenous tirofiban bolus administration to patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment.
Methods: Selected 90 patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment, randomly divided into the intracoronary group (intracoronary tirofiban 10, 30 cases), intravenous group (intravenous tirofiban 10 μg/kg bolus then 0.15 μg.kg-1.min-1 intravenous continuous infusion for 36 h, 30 cases) and control group (without tirofiban, 30 cases). The level of PMPs were assessed before tirofiban infusion, at 10 min and 24 hours after tirofiban infusion, and at 12 hours after stopping tirofiban infusion by the flow cytometry. Clinical and angiographic features were recorded and analyzed.
Results: There was no significant difference in baseline of PMPs between intracoronary group, intravenous group and control group (P>0.05). The level of PMPs were significantly lower in intracoronary (3.6%±2.3%) and intravenous group

(5.1%±2.7%) compared with control group (6.7%±3.2%) (P <0.01 VS P=0.04) at 10 mins after tirofiban infusion. The PMPs were also lower in intracoronary group than intravenous group (P=0.02). At 24 hours after tirofiban infusion, the PMPs of intracoronary and intravenous group was similar (P>0.05) and was significantly higher than control group (P=0.01 VS P =0.03) .PMPs was similar at 12 after stopping tirofiban use among the 3 groups (P>0.05) .Intracoronary group were superior to intravenous group and control group in terms of TIMI flow grade (P=0.03 VS P<0.01) and TIMI myocardial perfusion grade (P=0.02 VS P<0.01) immediately after PCI. MACEs rate in intracoronary group was lower than control group (P=0.03) .And MACEs rate between intracoronary group and intravenous group, intravenous group and control group were similar (P>0.05, respectivly). The incidence of bleeding events among 3 groups was similar.

Conclusions: Intracoronary tirofiban compared to the intravenous group, can effectively reduce the number of PMPs in patients with acute ST-segment elevation myocardial infarction undergoing emergency interventional treatment, achieve the purpose of the inhibition of activated platelets quickly, and reduce total MACEs events rate, but did not increase the risk of bleeding.

GW25-e0832

TNF receptor-associated factor 6 (TRAF6) mediates the angiotensin-induced non-canonical TGFβ pathway activation and differentiation of c-kit+ cardiac stem cells

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Objectives: TNF receptor-associated factor 6 (TRAF6) acts as a multifunctional regulator of the transforming growth factor (TGF) -β signaling pathway, and mediates Smad-independent JNK and p38 activation via TGF-β. This study was performed to test the hypothesis that TGF-β/TRAF6 is essential for angiotensin-II (Ang II) -induced differentiation of rat c-kit$^+$ cardiac stem cells (CSCs) .

Methods: c-kit$^+$ CSCs were isolated from neonatal Sprague Dawley (SD) rats, and their c-kit status was confirmed with immunofluorescence staining. A TGF-β type I receptor inhibitor (SB431542) or the small interfering RNA (siRNA) -mediated knockdown of TRAF6 were used to investigate the role of TRAF6 in TGF-β signaling. Rescue of TRAF6 siRNA transfected cells with a 3'UTR deleted siRNA insensitive construct was conducted to rule out the off target effects of the siRNA. TRAF6 dominant negative (TRAF6DN) vector was constructed and used to infect c-kit$^+$ CSCs, and western blotting was used to assess the expression of TRAF6, JNK, p38, cardiac-specific proteins, and Wnt signaling proteins. Physical interactions between TRAF6 and TGFβ receptors were studied by coimmunoprecipitation.

Results: Cardiac differentiation was suppressed in the absence of TRAF6. Forced expression of TRAF6 enhanced the expression of TGF-β-activated kinase1 (TAK1), and inhibited Wnt signaling. Furthermore, TRAF6 increased the expression of cardiac-specific proteins (cTnT and Cx-43) but inhibited the expression of Wnt3a.

Conclusions: Our data suggest that TRAF6 plays an important role in Ang II induced differentiation of c-kit+ CSCs via the non-canonical signaling pathway.

GW25-e1647

Effects of Herbal medicine Aconite Compound on Heart failure

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Objectives: Herbal medicine, also known as Chinese medicine, plays a role in treatment of heart failure. Aconite, as one of Chinese medicine, is useful for treatment of heart failure. purpose of this study is to test efficacy of aconite compound in improving heart funciton from both clinical and animal study to provide reference for clinical application.

Methods: (1) Clinical study 41 Patients with chronic heart failure (CHF) were given treatment of Chinese medicine aconite compound (twice daily for 8 weeks). Before and after treatment, cardiac function (according to New York Heart Association NYHA classification method) and echocardiography (LVEF%) were observed; (2) Animal study SD rats (SPF, male, 240 - 280g), acute heart failure model was used as follows (isolated right carotid artery, heparin catheter was inserted through aortic valve into left ventricular cavity, intravenous injection of nimodipine (0.6 mg/kg), when left ventricular pressure maximal rate of rise (+LVdp/dtmax) decreased significantly, given treatment drugs, (Aconite compound dose as 7g/kg and duodenal administration, and Cedilanid 0.2mg/kg, femoral vein injection), continue tracings 1h, including before modeling, modeling immediately, after administration 10 mins, 20 mins, 30 mins, 40 mins, 50 mins, 60 mins to observe following indicators: left ventricular systolic pressure (LVSP mmHg), left ventricular end-diastolic pressure (LVEDP mmHg) .

Results: (1) 21 patients were male (44.9%), 20 patients were female (55.1%), minimum age is 53 years, the maximum age is 95 years, mean age 73.88 +1.343 (years old), P=0.24>0.05, normal distribution; there was improvement of heart function acording to before and after treatment echocardiography, the LVEF% Value was as follows: 44.71 +14.846 % VS 52.05 +14.854 %, t=-2.669, P=0.014<0.05; (2) Compared with normal group, LVEDP (-0.29±0.57 vs 1.96±0.99, P<0.05) were significantly higher, LVSP (105.37±6.39 VS 129.00±7.47, P <0.01) was significantly lower for acute heart failure group. After femoral vein injection cedilanid 0.2mg/kg, compared with the model group, LVEDP decreased significantly (-0.74±1.12 VS -0.53±1.18), LVSP significantly higher (107.73±7.49 VS 117.11±7.16) after the treatment at 10mins, 118.00±7.45 at 20 mins, 118.64±7.44 at 30mins, 120.05±8.25 at 40mins, 122.74±8.80 at 50 mins, 125.04±9.71 at 60mins), heart failure model copied successfully; Aconite compound 7g/kg were administered orally to acute heart failure rats, LVSP (105.37±6.39 in model group vs 112.36±5.83 at 10 mins, 115.69±6.29 at 20mins, 117.21±7.71 at 30mins, 118.34±10.99at 40mins, 120.08± 12.82 at 50mins, 123.96±11.95 at 60mins), LVEDP (1.96±0.99 VS 0.05±0.87 at 10mins, -0.00± 0.99 at 20 mins, -0.06 ±0.85 at 30mins, 0.02 ±0.84 at 40mins, 0.14±0.87at 50mins, 0.13±0.77 at 60 mins after the treatment) showed significant improvement (P<0.05;P<0.01).

Conclusions: Aconite compound can be used for the heart failure by improving hear function, and there is no significant side effects if it is used properly.

GW25-e4404

Cardiosphere and Cardiosphere-Derived Cells Can Be Derived from The Cadaver Autopsy

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Objectives: Currently, cardiosphere (CSP) and cardiosphere-derived cells (CDCs) are mainly obtained through myocardial biopsy and surgical, but there is limited access to tissue size, technical requirements and faced with infection, trauma and other problems. We assume that there is still a large number cardiac stem cell survival in the cadaver cardiac, we can get a sufficient amount of functional CSPs and CDCs through cadaver autopsy methods.

Methods: Mouse (C57BL/6) were sacrificed and placed in a refrigerator at 4 °C for 0 to 3 days, cardiac tissue was removed at different time points (D0, D1, D2, D3), cut into small pieces (approximately 1.5 cm3) and placed into dishes to culture explant-derived cells (EDCs) with complete explant medium. Flow cytometry analysis the expression of stem cell surface markers of EDCs such as CD117, CD133, CD105, Sca-1, CD90. EDCs were cultured with Cardiosphere growth medium to form CSP. CD105, CD117, GATA-4, Nkx2.5 and Connexin-43 were detected by immunofluorescence. CSP was placed in fibronectin covered flasks and grow into a layer of CDCs. Early cardiac transcription factors GATA-4, Nkx2.5 and Connexin-43 were detected by polymerase chain reaction (PCR), cardiac stem cell surface markers CD117 and Sca-1 were tested by flow cytometry. The proliferation ability was tested by cck-8, c-TnI and vWF were stained by immunofluorescence after induction of differentiation.

Results: Nine days after placing the cardiac tissue in dishes, EDCs from mouse cadaver cardiac were successfully isolated and cultured. Each group of EDCs are rich in stem cell surface markers expressing such as CD117, CD133, CD105, Sca-1, CD90, and could culture into CSP and CDCs. With the extension of the dead days, the number of EDCs was able to harvest from the autopsy gradually decreased, the amount of EDCs could harvest at D3 decreased significantly (the number of each 60 mm dishes at D0, D1, D2, D3 were $86.00\pm5.27\times10^4$, $66.92\pm3.15\times10^4$, $49.67\pm3.17\times10^4$, $23.75\pm1.52\times10^4$, respectively). There is no significant differences in CD117 and Sca-1 expression at D0 and D1 in EDCs and CDCs, but decreased at D2 and D3. No significant difference was found in the ability of EDCs to form CSPs (the number of each well of 24-well plate at D0, D1, D2, D3 were 38.33±1.25, 38.00±2.45, 37.33±1.25, 38.00±2.16, respectively), and each group of CSPs was positive expression CD117, CD105, GATA-4, NKX2.5 and Connexin-43. CDCs obtained from cadaver autopsy both express GATA-4, Nkx2.5 and Connexin-43 when detected by PCR and immunofluorescence, but decreased in D2 and D3. There is no significant difference in proliferation when detect by cck-8 and c-TnI, vWF expression after induction of CDCs.

Conclusions: There is a variety of stem cells survival in cadaveric cardiac even at three days post-mortem. EDCs harvested through cadaver autopsy methods can form enough CSPs and CDCs. CDCs from cadaver autopsy also have strong ability of proliferation and differentiation. CSPs and CDCs obtained from cadaver autopsy may be used as a source or alternative sources of allograft transplantation and needs further research in vivo, which may solve the insufficient source problems existing in cardiac stem cells.

GW25-e4537

Statin effects on UA patients microRNA expression profile and regulatory functional network analysis

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Objectives: Unstable angina (UA), an acute coronary syndrome caused by disruption of atherosclerotic plaque triggered thrombosis. The blood vessel narrow and reduction of blood flow induce the symptom. Statin therapy benefits UA patients by cholesterol independent effect. Yet the mechanism of statin pleiotropic effect remained to be study. MicroRNAs (miRNAs), small non-coding RNAs, are post-transcriptional regulators of gene expression. In this study we aim to investigate statins' novel mechanism mediated by miRNAs. Moreover we carry out systematic analysis of the miRNAs functional networks in atherosclerotic lesions.