Caffeine improves systemic lupus erythematosus endothelial dysfunction by promoting endothelial progenitor cells survival

Keywords: Systemic lupus erythematosus, Diet and nutrition, Cardiovascular disease

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Background: Circulating endothelial progenitor cells (EPCs) are widely demonstrated biomarkers of endothelial function. Their frequency and function varied in systemic lupus erythematosus (SLE) patients, with a significant association with subclinical atherosclerosis [1]. Caffeine, one of the most widely consumed products in the world, seems to improve endothelial cells number and EPCs migration in coronary artery disease both in mouse models and in patients [2]. We recently demonstrated the impact of caffeine on SLE disease activity status, in terms of SLE Disease Activity Index 2000 (SLEDAI-2K) values and serum cytokine levels [3].

Objectives: The aim of this study was to evaluate the possible role of caffeine intake on endothelial function in SLE patients, by assessing its effect on number and function of EPCs both ex vivo in SLE patients and in vitro in healthy donors (HD) treated with SLE sera.

Methods: We performed a cross-sectional study enrolling SLE patients, excluding patients with history of traditional cardiovascular risks factors. Caffeine intake was evaluated using a 7-day food frequency questionnaire. At the end of questionnaire filling blood samples were collected from each patient to assess circulating EPC percentage. EPCs were detected by using a flow cytometry analysis defined as KDR CD34 double positive cells. Subsequently, EPCs pooled from HD were co-cultured with caffeine at 0.5 mM and 1 mM with and without SLE sera. After 7 days, we evaluated the cells morphology and the ability to form colonies; moreover, we analyzed for the percentage of annexin V-positive (AV) apoptotic cells by flow cytometry analysis and for levels of autophagy and apoptotic markers LC3-II, p62 and Bcl2 by western blot, alone or in the presence of protease lysosomal inhibitors E64d and Pepstatin A. Finally, we performed a WB analysis to assess the A2AR/SIRT3/AMPK pathway.

Results: We enrolled 31 SLE patients (F:M 30:1, median age 43 years, IQR 18; median disease duration 144 months, IQR 180). We found a EPCs median percentage of 0.03% (IQR 0.04) observing a positive correlation between caffeine intake and circulating EPCs percentage (p=0.03, r=0.4). Moving on in vitro experiments, HD EPCs treated with SLE sera and caffeine showed an improvement in morphology and number of EPCs-CFU in comparison with those incubated without caffeine (p=0.0003). The colonies treated with SLE sera were poorly organized in comparison with HD; the addition of caffeine restored the colony structure. After treating HD-EPCs with SLE sera we observed an increase in AV positive cells and p62 values and a reduction of LC3-II and Bcl2 values; the addition of caffeine was able to significantly reduce AV positive cells and p62 values and to significantly increased LC3-II and Bcl2 values, without any significant differences between caffeine 0.5 mM and 1 mM (Figure 1 A-D). After E64d and pepstatin A treatment, both LC3II and p62 trend didn’t change, compared to untreated cells. Finally, we observed after caffeine treatment, in comparison with SLE sera alone, a significantly reduction in A2AR levels leading to an increase in protein levels of SIRT3 and subsequently AMPK phosphorylation (Figure 1 E-G).

Conclusion: We demonstrated, for the first time, a protective role of caffeine on endothelial function in SLE patients. Caffeine intake positively correlated with the percentage of circulating EPCs in SLE patients; moreover, caffeine in vitro treatment was able to improve EPCs survival and vitality through the inhibition of apoptosis and the promotion of autophagy via A2AR/SIRT3/AMPK pathway.

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Background: Pulmonary arterial hypertension (PAH) is one of the most important complications that seriously threatens the prognosis of patients with systemic lupus erythematosus (SLE), with complicated and unclear pathogenesis.

Objectives: Based on genomic studies and functional experiments, we aim to investigate candidate biomarkers and targeted therapy for the early diagnosis and timely treatment of SLE-PAH patients.

METHODS: 1) In order to screen susceptible genes of SLE-PAH, a number of 150 peripheral blood from SLE-PAH patients were subject to whole-exome sequencing (WES), and genome-wide association study (GWAS) was performed by comparing with 934 healthy controls. 2) The transcriptional expression levels on peripheral blood of SLE-PAH patients were examined by RT-qPCR to further evaluate the possible pathogenesis of the above screened genes. 3) Intervention experiments on human pulmonary artery endothelial cells (hPAEC) were performed to figure out the potential pathogenesis of the selected gene in vitro. RNA-seq and gene ontology were applied to identify the downstream pathways. 4) Established by pristane injection and hypoxia induction, SLE-PAH mice model was used to evaluate the pathogenicity and therapeutic value of selected gene. Pulmonary arterial pressure (PAP) was measured by right heart catheterization after tail-intravenous injection of therapeutic vectors.

RESULTS: 1) The tumor necrosis factor receptor-associated factor 5 (TRAF5) was identified as a susceptible gene of SLE-PAH based on WES and GWAS. 2) The significant reductions of TRAF5 on transcriptional level in peripheral blood of SLE-PAH patients were identified, indicating clinical diagnosis values. 3) Knockdown of TRAF5 significantly increased early apoptosis of hPAEC and triggered the pathogenesis of PAH through distinct pathways. 4) SLE-PAH mouse model was successfully established since they showed lupus phenotype and the mean PAPs were measured as over 40 mmHg. Tail-intravenous injection of TRAF5-overexpression vector attenuated PAH.

Conclusion: Lack of TRAF5 triggers the pathogenesis of PAH in SLE patients through inducing hPAEC abnormality. It is a susceptible gene of SLE-PAH and could be a candidate marker for diagnosis and therapy for SLE-PAH patients.

Keywords: Genetics/epigenetics, Systemic lupus erythematosus, Lungs

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Figure 1. A) Genomic and protein simulation structure TRAF5 (a susceptible gene of SLE-PAH). Red dots represent mutation sites that screened from SLE-PAH patients. P.G468R mutation causes dysfunction of protein. B) TRAF5 mRNA expression levels in PBMC of healthy-controls, SLE-PAH and SLE-nPAH patients. *p<0.05. C) shTRAF5 transfected human PAEC. The transfection efficiency reaches 80% (The percentage of EGFP positive cells in all cells of bright field) when MOI=20. All three knockdown vectors of shTRAF5 showed significantly down regulation of TRAF5. *p<0.05, **p<0.01 . D) FACS was performed to detect early apoptotic cells labeled with Annexin V-APC (Apoptosis Detection Kit). The group of shTRAF5 showed significantly increased apoptosis compared with groups of control/shScramble. *p<0.05 E) Wound healing experiments were performed in different groups, and the distances of scratches at the same area in each group were measured at the time point of 0h, 6h, 24h, 48h. Abnormal migration was observed in shTRAF5 transfected PAECs.

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