experimental lupus model and the effect of ER stress inhibition by 4-PBA on the frequency and function of Treg.

Methods: Murne lupus model were induced with female BALB/c mice at 7 or 8 weeks of age through Toll-like receptor (TLR) agonist treatment for 4 weeks. From the 8th week, mice were treated with phosphate-buffered saline (PBS), 4-PBA (500 mg/kg, three times weekly) and dexamethasone (1 mg/kg, once a day) for 4 weeks. The increment of body weight, spleen weight, anti-double-stranded DNA (anti-dsDNA) antibody titer, serum cytokine level and the pathology of glomerulonephritis were analyzed at 12 weeks of age. The population of immune cellular subset including activated T, B lymphocyte and Treg and suppressive functions of Treg were measured.

Results: 4-PBA significantly decreased the level of anti-dsDNA antibodies, serum TNF-α in murine lupus model, and which were comparable with the efficacy of dexamethasone. A significant decrease in accumulation of immunoglobulin, glomerulonephritis score was also observed in 4-PBA-treated and dexamethasone-treated mice compared with vehicle-treated group. ER stress inhibition decreased the activated T and B lymphocytes population of splenocytes, but the population of Treg was not significantly different between vehicle group and 4-PBA group. However, there was the markedly enhanced suppressive capacity of Treg in 4-PBA-treated group.

Conclusions: The results suggest that ER stress inhibition attenuates disease activity in experimental model, especially in nephritis through improving the suppressive capacity of Treg. Thus, reduction of ER stress could be used as a beneficial therapeutic strategy in SLE.

Disclosure of Interest: None declared

Results: LncMKLN1, dominantly located in nucleus, was up-regulated in lupus patients compared to healthy donors, and could be induced by IFNα and TLR ligands in HMRC. Silencing lncMKLN1 significantly reduced the expression of a group of interferon-inducible genes, including IFIT3, OAS1, CXCL10. etc. We used lose-of-function and gain-of-function strategy through CRISPR system to confirm that IncMKLN1 positively regulated type I interferon pathway. Furthermore, it was identified the involvement of IncMKLN1 in interferon signalling pathway was through regulating the expression of STAT1, IRF9 and phosphorylation of IRF9 and STAT1 although its mechanism is also needed to investigate.

Conclusions: Upregulated IncMKLN1 expression contributed to abnormal activation of interferon pathway of SLE.

Disclosure of Interest: None declared

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Systemic sclerosis, myositis and related syndromes – etiology, pathogenesis and animal models

Background: Mesenchymal stem cells (MSCs) exhibit promising therapeutic potential in systemic lupus erythematosus (SLE). Increased apoptotic cells (ACs) were observed in SLE. In our previous study showed: MSC transplantation reduced AC levels in patients with SLE. Yet the effects of ACs on MSCs are not clear.

Objectives: This study aims to investigate the effects of ACs on MSCs and efficacy of AC treated MSCs (AC-MSCs) in SLE.

Methods: Jurkat T cells were irradiated by ultraviolet light to induce apoptosis and then co-cultured with umbilical cord derived MSCs. Then, ACs were removed and MSCs were further co-cultured with human peripheral blood mononuclear cells (PBMCs). The inhibition of MSCs on PBMC proliferation was detected by flow cytometry. MSCs and AC-MSCs were infused into lupus prone MRL/lpr mice respectively to compare their therapeutic effects.

Results: The suppression of MSCs on PBMC proliferation was significantly enhanced after co-culture with ACs. In vivo study showed that AC-MSCs significantly increased the survival rate of MRL/lpr mice and decreased urine protein as early as one week after treatment, while MSCs decreased urine protein eight weeks post infusion. Moreover, AC-MSCs remarkably reduced the number of splenic plasma cells and serum anti-dsDNA levels, whereas MSCs only showed decreased tendency.

Conclusions: ACs enhanced therapeutic effects of MSC transplantation in lupus mice, which provides new insights into MSC modification in the treatment of SLE.

Disclosure of Interest: None declared


Vitamin D and vitamin D receptor in patients with scleroderma subtypes

Background: Scleroderma is an autoimmune multisystemic connective tissue disease characterised by skin and internal organ fibrosis.1 Underlying mechanism is still unclear for SSc. Besides there is no specific treatment for SSc, various treatments may alleviate symptoms and improve the quality of life. Epigallocatechin-3-gallate (EGCG) is a phenol with antioxidant effects in many disease processes.2 In this disease, oxidative stress may play a role for pathogenesis.2 Recent studies showed a relationship between oxidative/antioxidative markers and SSc.3

Objectives: The aim of this study was to investigate the antioxidant effects of epigallocatechin-3-gallate in the scleroderma process in experimental mouse model with bleomycin.

Methods: Thirty-two healthy female Balb-c mouse species were used and randomly divided into four groups: control, bleomycin, bleomycin + EGCG, EGCG. At the end of the experiment, skin tissues were collected. Sodium dismutase enzyme (SOD) and malondialdehyde (MDA) levels have been analysed for oxidative stress. High performance liquid chromatography (HPLC) was used for MDA measurements. Colorimetric kit was used for SOD analysis. Furthermore, the ratio of phosphorylated p-38/total p-38, and phosphorylated Akt/total Akt protein and NF-kappa B were measured by western blotting. Immunohistochemistry (u-SMA), histochmistry (masson trichrome-hematoxylin and eosin) studies were also performed on FFPE skin samples.

Results: When the experimental and control groups were compared, the degree of fibrosis in the connective tissue of the dermis areas stained with masson trichrome decreased in the EGCG group. SOD activity was increased in the EGCG groups compared to the positive control group, and MDA was significantly decreased in the EGCG groups. According to Western blotting results, p-38 MAPK and NF-κB were found to decrease significantly in the EGCG groups compared with the controls. Parallel to these findings, phosphorylated Akt protein was found to increase in the EGCG groups compared with the control groups.

Conclusions: It has been shown that EGCG can antioxidantive effect in scleroderma.

REFERENCES:

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MOLECULAR MECHANISMS MEDIATING ANTIOXIDANT EFFECT OF EPIGALCATECHIN-3-GALLATE IN EXPERIMENTAL SCLERODERMA MODEL

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Background: Scleroderma (SSc) is an autoimmune multisystemic connective tissue disorder. SSc patients demonstrated high oxidative stress and high levels of inflammatory cytokines.1 Recent studies showed a relationship between oxidative/antioxidative markers and SSc.2

Objectives: The aim of this study was to investigate the antioxidant effects of epigallocatechin-3-gallate in the scleroderma process in experimental mouse model with bleomycin.

Methods: 19 SSC patients and 6 healthy controls were included in the study and they were classified according to the 2013 ACR/EULAR criteria. They were applied to Dokuz Eylul University. Faculty of Medicine, Department of Rheumatology-Immunology, between 2015–2017. Rodnan scores were calculated for all scleroderma patients. 11 were of the limited type and 8 were of the diffuse type of scleroderma. Informed consents were obtained from all participants. 1 ml of total blood was collected. Vitamin D levels were determined in serum.

VDR gene expression was determined by quantitative PCR in isolated RNAs from the blood. Changes in mRNA levels were analysed according to the ΔΔCT method and beta-actin was used as the housekeeping gene. Student-t test was used as a statistic. In addition, Pearson correlation test was used to determine the relationship between Rodnan score and VDR gene expression.

Results: VDR gene expressions in diffuse type scleroderma patients were statistically significantly decreased compared to the control (p<0.01). It was found that VDR gene expression in limited type scleroderma patients did not show any significant difference when compared to control (p=0.16).

Also, Vitamin D levels and vitamin D expressions were no correlation in scleroderma subtypes (p=0.2)

Conclusions: VDR gene expression decreased in patients with diffuse type scleroderma and showed negative correlation with Rodnan score. Further studies are planned to increase the number of samples to obtain more information.