SPECIAL SESSION

SSc VASCULO path VASCULITIS

SSc 1 DECREASE ACTIVITY OF DNA DEMETHYLASE IN SSC FIBROBLAST AND MICROVASCULAR ENDO THELIAL CELLS: A POSSIBLE MECHANISM FOR PERSISTENT SSC PHENOTYPE

B. Kahaleh1 and W. Wang1
1Department of Medicine, University of Toledo, Toledo, OH, USA.

Objectives. DNA methylation is one of the best-characterized epigenetic modifications that have been implicated in numerous biological and pathological processes. It is initiated by DNA methyltransferases (DNMTs) 3a and 3b and maintained by the maintenance enzyme DNMT1. DNA methylation is now believed to be a dynamic process as active DNA demethylation has been observed. Thus, during cellular division a competition between DNMT1 and DNA demethylase determine the maintenance or the reversal of DNA methylation in the daughter cells. The molecular identity of DNA demethylase remains elusive but its activity can be measured by functional assay. DNMT1 expression is up-regulated in SSc microvascular endothelial cells (MVECs) and fibroblasts (FBs) in association with persistence of DNA methylation of the CpG islands in the promoter region of key underexpressed genes (i.e. Fli1 and eNOS). Thus, in this study, we thought to examine the activity of DNA demethylase in SSc and control cells and the role of microRNA (miRNA) in the regulation of DNA demethylation activities.

Methods. MVECs and FBs were isolated from involved SSc skin and control subjects. DNMT1 expression levels were determined by qRT-PCR and western blots. DNA demethylase activity was measured in nuclear extracts using the EPI Quik™ DNA Demethylase Activity/Inhibition Assay Kit. Small RNA molecules including miRNA were isolated from SSc and control MVECs and FBs using PureLink™ miRNA isolation kit. The effects of miRNA on DNA demethylase activity was examined in SSc and control cells by transfecting the cells with SSc and control miRNA.

S.S.5 ROLE OF ENDOPLASMIC RETICULUM STRESS IN THE PATHOGENESIS OF SCLERODERMA-ASSOCIATED INTERSTITIAL LUNG DISEASE

G. Bogatkevich1, T. Akter1, I. Atanelishvili1, J. Liang1, D. Spyropoulos1 and R. Silver2
1Medical University of South Carolina, Charleston, USA.
2Department of Medicine, University of Toledo, Toledo, OH, USA.

Background. The endoplasmic reticulum (ER) is recognized as the primary site of synthesis and folding of secreted, membrane-bound and some organelle-targeted proteins. Stresses such as hypoxia, oxidative stress and defective protein secretion induce ER stress, leading to impaired post-translational processing of secretory proteins that accumulate within the cell and triggering cytotoxic proteolytic unfolded protein response (UPR). If the UPR fails to control the cytotoxic effects of these accumulated proteins, pro-apoptotic signals are activated and the cell will undergo apoptosis. Recently, it has been reported that ER stress and apoptosis of alveolar epithelial cells (AECs) are prominent features in idiopathic pulmonary fibrosis. Here we demonstrate that ER stress also plays a significant role in the pathogenesis of scleroderma-associated interstitial lung disease SSc-ILD.

Materials and methods. Lung tissues were collected postmortem from controls and from SSc patients who fulfilled the American College of Rheumatology preliminary criteria for SSc and had evidence of lung involvement. The diagnosis of SSc-ILD was confirmed by histological examination of postmortem lung tissue. Additionally, lung tissue was obtained from mice with bleomycin-induced pulmonary fibrosis and from control animals. Lung fibroblasts and AECs were isolated using standard procedures. Immunohistochemistry was performed using Vectastain ABC kit from Vector Laboratories and Histostain-SP kit from Invitrogen. Apoptosis was measured by Cell Death Detection ELISA from Roche Diagnostics.

Results. We observed staining for the pro-apoptotic ER stress marker C/EBP homologous protein (CHOP) exclusively in AECs surrounded by fibrotic tissue, or localized in thickened alveolar septa in SSc-ILD patients and in bleomycin-treated mice, but not in normal lung tissue from human or murine controls. In contrast, myofibroblasts, positively stained for α-SMA, did not show notable immunoreactivity for ER stress markers. We found that thymobin, known to be elevated in SSc-ILD patients and in the bleomycin murine model of I LD, has no observable effect on CHOP expression in lung fibroblasts; however, it up-regulates the expression of CHOP in primary AECs and in AS49 cells via an Ets1-dependent pathway. Importantly, lung myofibroblasts are no longer protected by thymobin or TGF-β from apoptosis, i.e. they lose their resistance to apoptosis, when transfected with CHOP.

Conclusions. UPR and ER stress is evident in AECs of patients with SSc-ILD and in AECs of a murine model of I LD. The ER stress marker CHOP is involved in regulation of apoptotic mechanisms in fibrotic lungs downstream of thymobin and TGF-β, making it a possible novel target for the treatment of SSc-ILD.
Results. (i) DNM1T expression levels were significantly up-regulated in SSc cells (mean 3.2- and 2.8-folds in MVECs and FBs, respectively, vs control cells, mean 3 cell lines each); (ii) DNA demethylase activity was significantly reduced in SSc cells (42 and 51% in MVECs and FBs, respectively, vs control cells, mean 3 cell lines each); (iii) The knockdown of DNM1T using DNM1T-specific siRNA did not affect demethylase activity. (iv) Transfection of SSc cells with control miRNA resulted in decrease expression of DNM1T and increase activity of DNA demethylase, while the transfection of control cells with SSc miRNA resulted in up-regulation of DNM1T and reduced DNA demethylase activity. (v) SSc MVEC and FB transfection with control miRNA normalized abnormal gene expression profile.

Conclusions: This study demonstrates up-regulation of DNM1T and diminished activities of DNA demethylase in SSc cells and that DNA demethylase activity is regulated by miRNA.

SS.1.2 INTENSE PULSED LIGHT IS AN EFFECTIVE TREATMENT FOR SSc-RELATED TELANGIECTASES, AS ASSESSED BY LASER DOPPLER IMAGING

T. Moore1, A. Murray2, H. Richards3, H. Ennis1, C. Griffiths1 and A. Herrick1
1School of Translational Medicine, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK and 2Department of Clinical Health Psychology, Mercy University Hospital, Cork, Ireland.

Introduction. Telangiectases are a very visible representation of the microvascular changes inherent in the SSc disease process and cause significant psychological distress, particularly when occurring on the face. Current treatments include camouflage techniques and laser therapy. However, there is often a poor outcome from laser therapy; many SSc-related telangiectases do not respond, and those which may recur. Adverse effects of laser treatment include purura, swelling and scarring. Therefore, there is a need for new approaches to therapy. Intense pulsed light (IPL) has been shown to have successful outcomes in non-SSc-related telangiectases. We performed an open pilot study to examine the efficacy, safety and tolerability of IPL treatment for SSc-related telangiectases. Here we report treatment response as assessed by two imaging modalities: laser Doppler imaging (LDI) and thermography.

Methods. Patients underwent three treatments of IPL at monthly intervals. Patients then attended for follow-up visits at 1, 6 and 12 months after the final treatment. LDLI and thermography were performed at each visit.

Results. Twenty patients median (range) age 58 (37–69) years; 4 males, 16 females; 18 lScSSc, 2 dcSSc) with telangiectases on their face and/or upper limbs were recruited in to the study. The sites selected for treatment were: 12 cheeks, 1 forehead, 2 upper arms/shoulders and 5 hands. Seventeen patients completed the study. Perfusion as measured by LDI (in arbitrary units) was significantly reduced, compared with baseline [median (IQR) 2.66 (13–52)], at 1 month [1.70 (0.63–3.39), P = 0.004] and at 6 months [P = 2.05 (0.78–3.19), P = 0.004] post-treatment, but not at 12 months [1.61 (1.02–4.87), P = 0.130]. Individual telangiectases could not be resolved in thermal images. Instead, the whole area of treatment was selected for treatment with IPL. No difference was found in skin temperature (◦C) between baseline [32.6 (30.5–36.6), 1 month [32.5 (25.4–36.1), P = 0.353], 6 months [33.4 (25.5–35.6), P = 0.845] and 12 months [34.0 (25.5–34.7), P = 0.915] visits.

Conclusions. In this pilot study (the first of IPL treatment in SSc), most patients improved after IPL treatment as assessed by LDI at 1 month and 6 months following treatment. However, the degree of improvement was not maintained in all patients suggesting that further treatments might be necessary. Longer term studies of this novel treatment approach are now required. Our results suggest that LDI, which gives a direct measure of perfusion, is likely to be a key outcome measure in future clinical trials of telangiectases.

SS.1.3 ANGIogenic BIOMarkers PREDICT THE DEVELOPMENT OF DIGITAL ULCers In SSc Patients

J. Avouac1, C. Meune2, A. Kahan3, G. Chiocchia4 and Y. Allarone1
1Rheumatology A Department, 2Cardiology Department and 3INSEMr U1016 and CNRS UMR8104, Paris Descartes University, Cochin Institute, APHP, Paris, France.

Background/Purpose. To evaluate the possible merit of different endothelial markers for the prediction of ischaemic digital ulcers (DUs) and other microvascular complications.

Method. Endothelial markers were assessed in a prospective cohort of 100 SSc patients without known cardiovascular involvement at presentation. Circulating endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) were quantified in peripheral blood by flow cytometry. Serum levels of PGF, sVCAM and VEGF were measured by quantitative sandwich ELISA technique. The primary outcome was the occurrence during a planned 3-year period of any new ischaemic DU. Secondary endpoints were the occurrence of at least one at cardiac/vascular event, assessed by an exploratory composite index defined by the occurrence of at least one of the following events: (i) one or more new ischaemic DUs; (ii) pre-capillary pulmonary hypertension (PH); (iii) left ventricular (LV) dysfunction [LV ejection fraction (EF) <50%]; or (iv) scleroderma renal crisis (SRC).

Results. The mean (s.d.) age of the 100 patients (69 women) was 56 (13) years old and the mean (s.d.) disease duration was 9 (8) years at baseline. During the planned follow-up, 17 patients developed new ischaemic DU (10 patients with a history of previous DU and 7 with no previous DU). Regarding other vascular complications, PH occurred in five patients, LV dysfunction in four and SRC in a single patient. By univariate analysis, low EPC counts (P = 0.009), high PGF (P = 0.007) and sVCAM (P = 0.04) serum levels were identified as predictive biomarkers of the occurrence of at least one new DU. Multivariate analysis including these three biomarkers and SSc-related disease characteristics identified high PGF serum levels (HR = 5.04, 95% CI 1.19, 21.09) and a history of DU (HR = 9.51, 95% CI 1.54, 58.77) as independent predictors of new DU. In an alternate model excluding patients with a history DU at baseline, low EPC counts (HR = 7.95, 95% CI 2.09, 30.93) and high PGF serum levels (HR = 13.46, 95% CI 1.58, 114.73) were found as predictors of new DU. Regarding secondary outcome, low baseline EPC counts (HR = 4.56, 95% CI 1.04, 20.06, P = 0.03) and elevated PGF serum levels (HR = 5.04, 95% CI 1.42, 24.15, P = 0.02) were independent predictors in multivariate analysis of the occurrence of cardiac/vascular events.

Conclusion. This study identified low circulating EPC counts and high PGF serum levels as new predictors of new DU in SSc. It highlights the critical role of angiogenesis in this vascular outcome. These markers may improve DU risk stratification and therefore allow earlier therapeutic intervention.

S.3.1 WATERMELON STOMACH IN SSc: A EUSTAR CASE-CONTROL STUDY

1Rheumatology A Department, Paris Descartes University, Cochin Hospital, Paris, France, 2Jefferson Medical College, Philadelphia, USA, 3Rheumatology and Clinical Immunology, Brescia, Italy, 4University of Michigan, Ann Arbor, USA, 5University of Milan, Milan, Italy, 6Department of Clinical Medicine, La Sapienza University, Rome, Italy, 7Rheumatology Unit, Department of Medicine, Verona, Italy, 8University of Rome, Medical Clinic and Therapy Department, Rome, Italy, 9Division of Rheumatology, Department of Medicine, Geffen School of Medicine, University of California at Los Angeles, LA, USA and 10Georgetown Univ Medical Center, WA, USA.

Background/Purpose. Watermelon stomach (WS) or gastric antral vascular ectasia (GAVE) is a very rare gastric complication of SSc. GAVE seems to be a component of the general microangiopathy that characterizes the disease and it is thought as a severe complication. Despite the large use of gastroscopy, the prevalence of SSc-GAVE

SESSION 3

THE GUT