OP0037 FIBRINOLYTIC SYSTEM AND ACE I/D POLYMORPHISM IN SYSTEMIC SCLEROSIS

¹C Angotti, ²F Gensini, ²C Fatini, ³B Battaglini, ³E Sticchi, ⁴S Fedi, ¹A Righi, ⁵G Pepe, ⁴R Abbate, ¹M Maticucci Cerinic. ¹Department Internal Medicine, Section of Rheumatology; ²Department Clinical Physiopathology, Sect of Gen; ³Department Medicine and Surgery; ⁴Thromb Cent, University of Florence, Florence; ⁵Department Internal Medicine, University Tor Vergata, Rome, Italy

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Background In Systemic sclerosis (SSc), an imbalance of the fibrinolytic system was shown. *In vivo* and *in vitro* studies suggest a role for Renin Angiotensin System in the regulation of fibrinolytic balance: Angiotensin II (Ang II) increases the plasminogen activator inhibitor²1 (PAI-1) production and secretion, and ACE reduces t-PA production. A polymorphism in the ACE gene consisting of an insertion or deletion (I/D) of 287 bp fragment in intron 16 was described. The D allele of ID polymorphism is associated with an increased level of ACE and ACE gene polymorphism may influence the production of Ang II.

Objectives To investigate possible alterations in the fibrinolytic system and to examine the role of ACE I/D polymorphism in SSc.

Methods PAI-1 activity, t-PA and D-Dimer plasma levels were measured in 61 SSc patients (36 limited, 25 diffuse) and 108 healthy controls. ACE I/D polymorphism was genotyped by PCR. PAI-1 activity and t-PA levels were assayed by chromogenic method and D-Dimer levels by ELISA.

Results A significant difference in ACE genotype distribution ($\chi^2 = 9.79$ $p = 0.007$) and allele frequency was observed between patients and controls (0.64 vs 0.50 $p = 0.009$). In limited SSc a higher frequency of ACE D allele was found (0.68 vs 0.56 in diffuse SSc $p = 0.23$). By univariate analysis an association between ACE D allele and limited, but not diffuse SSc, was found (OR DD+ID/II = 6.44 95% CI 1.45–28.54 $p = 0.005$ and OR DD+ID/II = 1.91 95% CI 0.60–6.08 $p = 0.31$ respectively). Median values of PAI-1, t-PA and D-Dimer were 6.2 U/ml (range 1–37.5), 10.5 ng/mL (range 4.3–28.1) and 156 ng/mL (range 64–1064, respectively. Patients had higher t-PA and D-Dimer mean levels than controls ($p = 0.000001$ and $p = 0.02$, respectively). No difference was observed in PAI-1 plasma levels between patients and controls ($p = 0.85$). No association between high PAI-1, t-PA and D-Dimer plasma levels and ACE D allele was observed.

Conclusion These data suggest that ACE D allele is highly associated with limited SSc. Thus, we could hypothesise that this allele may represent a risk factor for the development of limited SSc. Other mechanisms than the alterations of fibrinolytic system may have a role in determining the microvascular damage.

OP0038 AUTOLOGOUS HEMATOPOIETIC STEM CELLS (HSC) TRANSPLANTATION IN SCLERODERMA AND MYOSITIS

DC Farge, S Zohar, MH Andre, J Cabane, E Hachulla, P Philippe, J Sibilia, P Roblot, J Piette, S Chevrete, JP Marroveau, E Gluckman. Internal Medicine, Saint Louis, Paris, France

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Background Autologous bone marrow transplant is a new treatment for autoimmune diseases (AD).

Objectives To determine the feasibility, tolerance and efficacy of autologous HSC transplantation with CD34⁺ selection in severe scleroderma (Sc) and myositis (M).

Methods We designed a national, non randomised, open, phase I-II trial.¹ Mobilisation of HSC used cyclophosphamide (CYCLO) (4 g/m²) + G-CSF (5 microg/kg day) or G-CSF alone (10 microg/kg day) if left ventricular ejection fraction (LVEF) was < 40% until last apheresis. 9.5×10^6 CD34⁺/kg had to be collected in successive daily apheresis to obtain at least 2.5×10^6 CD34⁺/kg (Isoplex[®] 300i). Conditioning used CYCLO (200 mg/kg), or melphalan (140 mg/m²) if LVEF was < 40%, prior to CD34⁺ HSC reinfusion. Patients, haematological and immunological reconstitutions were recorded up to the end of aplasia and every 3 months thereafter. Sequential Bayesian analysis determined the rate of failure (either death or failure of mobilisation, selection or intensification) and results (median) were computed on a SAS program (6.0).

Results 12 Sc + 3 M, age 36 yrs, were included in 2 years, meanwhile 10 candidates died from their disease. HSC mobilisation was successful in 14 patients, but in 2 cases did not yield enough HSC to allow CD34⁺ selection. After 2 (1–4) aphereses, the total number of CD34⁺ collected for each patient was 10.9×10^6 /kg (3.9–34.3). Purity was 99.2% (92–99.8). CD34⁺ cells yield was 60.7% (46.4–80). Intensive immunosuppression was performed in 13 patients with low toxicity. Number of reinfused cells was 5.8×10^6 CD34⁺/kg (3.3–10.2). Duration of aplasia was 2.9 weeks (2.5–3) with low toxicity. No mortality was associated with the procedure. Early clinical evolution (18 mths) showed improved functional status (PS, SHAQ) and fall in skin score in 8/12 Sc and no clinical benefit in M.

Conclusion Autologous HSC transplantation is feasible in Sc and M, with a lower risk compared to spontaneous evolution of AD and early clinical benefit in Sc. These results call for phase III studies to compare cyclophosphamide alone versus autologous transplantation in Sc and for more myositis in phase I–II studies.

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OP0039 EVIDENCE OF THE INTRACELLULAR CYTOKINES IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH SYSTEMIC SCLEROSIS

¹C Naderio, ²K Esposito, ²A Farzati, ¹G Cuomo, ¹G Valentini, ²B Farzati. ¹Rheumatology Unit; ²Immunohematology Unit, Second University of Naples, Naples, Italy

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Background Systemic sclerosis (SSc) is currently considered a Th2 disease. The studies underlying such assumption have been mainly carried out on tissues. At present, no data has addressed this topic in the peripheral blood at a cellular level.

Objectives To investigate the activation of Th1 and Th2 cells in the peripheral blood of SSc patients.

Methods Peripheral blood mononuclear cells (PBMC) from 15 patients with SSc and 15 sex and age-matched healthy controls were investigated for intracellular IL-2, IL-4, IFN- γ . The assays were carried out on both unstimulated and PMA-activated PBMC incubated in the presence of 1 μ g/ml of ionomycin and 10 μ g/ml of brefeldin-A. Subsequently, samples were incubated with anti-cytokines antibodies and stained cells were analysed on a FACScan flow cytometer.

Results In basal conditions (i.e. unstimulated cells), 10 out of the 15 SSc patients versus 1 out of the 15 controls presented a significantly higher percentages (i.e. $> m \pm 2$ SD detected in

controls) of IL-2 positive cells; 6 patients and 1 control of IL-4 positive cells and 7 patients and 0 control of IFN- γ positive cells. Six patients presented increased percentages of both IL-2 and IL-4 and IFN- γ (i.e. they had both Th1 and Th2 activation), 4 of IL-2 positive cells and 1 IFN- γ only (isolated Th1 activation).

After PMA stimulation 6 patients presented increased percentages of IFN- γ positive cells; 1 of IL-4 and 0 patient of IL-2 positive cells.

Conclusion Our study demonstrates that Th1 activation occurs in peripheral blood of SSc patients. The role, if any, of such activation must be investigated.

OP0040 BUCILLAMINE AND ITS METABOLITES INDUCE SYNTHESIS OF VEGF DOSE DEPENDENTLY IN SYSTEMIC SCLEROSIS FIBROBLASTS VIA THE NF-KB PATHWAY

¹C Hagen, ¹O Distler, ²U Müller-Ladner, ³A Carossino, ¹RE Gay, ⁴R Nanagara, ⁵BA Michel, ⁶K Nishioka, ³M Matucci-Cerinic, ¹S Gay. ¹*Ctr Exp Rheum*; ²*Department Int Medicine I, University Hospital, Regensburg, Germany*; ³*Department Medicin Sect. Rheum., University Hospital, Florence, Italy*; ⁴*Department Rheum, University Hospital, KhonKaen, Thailand*; ⁵*Department Rheum, University Hospital, Zurich, Switzerland*; ⁶*Institute Medicine Science, St Marianna University, Kanagawa, Japan*

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Background Bucillamine (BUC) is an immunomodulatory drug used in the treatment of rheumatoid arthritis with inhibitory effects on B- and T-cell functions. However, the impact of Bucillamine on metabolic pathways of fibroblasts has not been addressed.

Objectives Since an altered metabolism of fibroblasts is a hallmark of systemic sclerosis (SSc), we investigated whether BUC and its metabolites SA981 and SA679 influence the cytokine pattern of skin fibroblasts and might be of therapeutical use in SSc.

Methods Cultures of skin fibroblasts from 6 patients with SSc and 5 healthy controls were incubated with BUC, SA981 and SA679 at different concentrations for 6 to 24 h. Quantification for VEGF, IL-6 and TGF- β 1 mRNA was performed with real-time PCR (TaqMan). Supernatants were analysed for VEGF by ELISA. In addition, fibroblasts were preincubated with the NF- κ B inhibitor pyrrolidine dithiocarbamate (PDTC) and analysed for the expression of VEGF as describe above. The trypan blue dye exclusion test was used to assess the viability of the cells. The effects on the chemotactic activity of fibroblasts were examined in a modified Boyden chamber assay.

Results VEGF mRNA was increased in all fibroblast cultures after BUC treatment compared to non-treated controls, whereas no effect was observed on the levels of IL-6 and TGF- β 1. VEGF mRNA increased dose dependently with both BUC and its metabolites. The most significant induction was observed after treatment with SA981 (1 mg/ml: 7.7 ± 4.3 , 0,6 mg/ml: 5.2 ± 2.3 ; 0,15 mg/ml: 2.9 ± 1.4 , 0,05 mg/ml: 2.5 ± 1.9 fold increase, $p < 0.05$). Fibroblast cultures from patients with SSc showed a consistent higher inducibility of VEGF than cultures from healthy controls (SSc: 9.9 ± 4.3 ; healthy controls: 5.2 ± 2.8 fold increase, $p < 0.05$). These results were confirmed on the protein level with a significant rise of VEGF in SSc cultures, but not in healthy controls. The increase of VEGF was not due to unspecific toxicity, since the cell viability was $>95\%$ in treated and non-treated fibroblasts. Preincubation with the NF- κ B inhibitor PDTC reduced the production of VEGF by 60%. Interestingly, there was a significant increase in the chemotactic activity of supernatants from fibroblasts treated with BUC (133 ± 39 cells/high power field = hpf), SA679 (72 ± 19 cells/hpf)

and SA981 (43 ± 5 cells/hpf) compared to non-treated controls (26 ± 7 cells/hpf).

Conclusion Our results indicate that BUC and its metabolites, in addition to the known immunomodulatory effects, may have favourable influence on neoangiogenesis in SSc through the release of VEGF by skin fibroblasts. Since impaired angiogenesis and inflammatory infiltrates are hallmarks in the pathogenesis of SSc, BUC may be of benefit in the treatment of this disease.

OP0041 ESOPHAGEAL MANOMETRY FINDINGS IN PATIENTS WITH RAYNAUD'S PHENOMENON AND POSITIVE ANTINUCLEAR ANTIBODIES

¹A Arteaga, ¹E Trujillo, ¹I Ferraz, ¹A Alvarez-Pío, ¹J Bethencourt, ¹F Díaz-González, ²C Casanova, ¹T González, ¹S Bustabad. ¹*Rheumatology*; ²*Digestive, Hospital Universitario de Canarias, La Laguna, España*

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Background

Objectives To study the esophageal manometry in patients with Raynaud's phenomenon and positive antinuclear antibodies (ANA) in the absence of esophageal, skin or internal organs manifestations.

Methods 14 patients, 13 women and 1 man (46,8 mean age; 9–65 years) with mean evolution time of Raynaud's phenomenon of 5 years were included in this study. Nailfold capillary microscopy study, autoantibodies profile and esophageal manometry (Synetics Manometry Unid. Synetics neumocapillary Pump and Poligraph) were performed in all patients. The lower esophageal sphincter, esophageal body and upper esophageal sphincter pressures were analysed independently.

Results 44% patients had a lower esophageal sphincter hipotonia which showed a significant relationship ($p < 0.05$) with the presence of both articular disease (33.5%) and anti-centromere antibodies (44.4%). However, the nailfold scleroderma pattern (61%) did not show a significant relationship with lower esophageal sphincter hipotonia. 33.3% patients showed unspecific motor alterations in the esophageal body. There was significant relationship ($p < 0.001$) between esophageal body alterations and the nailfold scleroderma pattern. In all patients studied, the function of the upper esophageal sphincter was within the normal limits. In the control group of patients with Raynaud's phenomenon and negative ANA, the esophageal manometry was normal.

Conclusion Alterations in the esophageal manometry are frequent in patients with Raynaud's phenomenon and positive ANA and they suggest the presence of systemic disease even in the absence of skin or internal organs involvement.

OP0125 OVEREXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS RECEPTORS IN SYSTEMIC SCLEROSIS (SSC): EVIDENCE FOR PROTECTIVE EFFECTS OF VEGF AGAINST THE DEVELOPMENT OF FINGERTIP ULCERS

¹O Distler, ²A Del Rosso, ³A Scheid, ¹J Rethage, ¹M Neidhart, ²A Carossino, ¹RE Gay, ⁴U Müller-Ladner, ⁵BA Michel, ³M Gassmann, ²M Matucci-Cerinic, ¹S Gay. ¹*Ctr Exp Rheum*; ²*Department Medicine Sect Rheum, University Hospital, Florence, Italy*; ³*Institute of Physiology*; ⁴*Department of Internal Medicine I, University Hospital, Regensburg, Germany*; ⁵*Department Rheumatology, University Hospital, Zurich, Switzerland*

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