S.5.6

THE PRESENCE OF A COLD TEMPERATURE SENSOR IN THE VASCULAR ENDOTHELIUM: ENHANCED EXPRESSION IN SSc SKIN AND ENDOTHELIAL CELLS DYSFUNCTION AFTER ACTIVATION

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Background. Cold exposure results in severe vasospasm and reperfusion vascular injury in SSc. The mechanisms responsible for enhanced cold sensitivity in SSc are poorly understood. Transient Receptor Potential Melastatin 8 (TRPM8) is a known cold sensing cation channel receptor. To date, TRPM8 expression has not been characterized in microvascular endothelial cells (MVEC). In this study we thought to investigate TRPM8 expression in normal and SSc MVEC and skin. We also investigated the effects of TRPM8 activation on MVEC gene expression.
Methods. MVEC were isolated from involved SSc skin and from matched healthy control subjects. The expression of TRPM8 was determined by RT-PCR, immunohistochemistry and by western blot analysis. TRPM8 activation was triggered by the addition of the agonist menthol or by exposure to cold temperature (18C°). The intracellular calcium concentration was determined by Ca2+ microfluorometry. The expression levels of TRPM8 in SSc-MVEC and SSc skin biopsies and the effects of TRPM8 activation on MVEC mRNA expression of ET1, NOS3 and PTGIS were determined by real time PCR.
Results. TRPM8 gene and protein expression in MVEC were confirmed by RT-PCR, Western blotting and immunohistochemistry. MVEC intracellular calcium ([Ca2+]i) influx into the cells in response to the addition of TRPM8 agonist menthol are demonstrated by Ca2+ microfluorometry studies. The activation of TRPM8 in MVEC by cold temperature or by menthol significantly increased the expression of ET1 (2.4 folds ± 0.21) and decreased NOS3 (62% ± 5.1 reduction) and PTGIS (61% ± 4.8) expression levels. These effects were reversed by the addition of the TRPM8 antagonist capsazepine. TRPM8 mRNA expression levels were significantly increased in SSc-MVEC (2.6 fold ± 0.22 vs. control MVEC) and SSc-skin biopsies (5.5 fold ± 2.3 vs. control skin biopsies).
Conclusions. The study demonstrates that human MVEC express functional TRPM8 and that there is increased expression of TRPM8 in SSc skin and in SSc-MVEC. TRPM8 may be involved in cold-induced vascular dysfunction through increase ET1, and decrease the NOS3 and PTGIS mRNA expression. The increased expression levels of TRPM8 in SSc-MVECs and SSc skin may mediate the known enhanced cold sensitivity in SSc. These results suggest that the blockade of TRPM8 activation could be an effective therapeutic strategy in SSc vasculopathy.

Session 7: *Treatment and DMARDS in SSc*

S.7.1

HOW TO TREAT RAPIDLY PROGRESSIVE SSc

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Systemic sclerosis (scleroderma; SSc) has a high mortality and morbidity but varies widely in rate of disease progression, reflecting clinical heterogeneity and disease subset. The most rapidly progressive cases are usually those with diffuse skin involvement and typically the maximum rate of progression is within the first 3 to 5 years of disease onset. The rate of change in skin scleroiss score can be assessed and has been associated with increased risk of major complications including cardiac involvement, lung fibrosis or scleroderma renal crisis. In many cases progression occurs over the first few months of disease and is associated with swelling and skin thickening affecting the distal limbs, pruritis over the proximal skin and the presence of tendon friction rubs on clinical examination. This constellation of signs is recognised to put a patient at high risk of scleroderma renal crisis and vigilant observation and patient education is important to minimise the delay in diagnosing this treatable complication that previously had very high mortality. Elevated ESR, platelet count and CRP are also recognised as markers of disease activity and poor outcome. In addition to skin progression there is risk of lung fibrosis and serious cardiac involvement with systolic impairment and cardiac arrhythmias. Thus treatment for SSc at this stage should include supportive management of manifestations such as Raynaud's phenomenon and reflux oesophagitis and investigation for major organ based pathology. Severe skin disease or presence of cardiac or lung fibrosis my require treatment with intravenous cyclophosphamide followed by maintenance immunosuppression with mycophenolate mofetil or methotrexate although less severe cases may be treated initially with these oral drugs, reserving cyclophosphamide for more severe or refractory patients. Finally, there are emerging data supporting the potential value of HSCT in this group. The challenge is case selection as the ASTIS trial suggests a potential treatment related mortality of up to 10% although long term survival sand disease burden may be significantly improved. Cases with cardiac involvement, pulmonary hypertension and smokers may be especially at risk of TRM and are probably not suitable cases for this treatment despite their poor overall prognosis with standard therapy. Autoantibody reactivity may be especially helpful in identifying cases of rapidly progressive diffuse SSc as the anti-RNA polymerase III and anti-U3RNP positive patients are more often in this group and ANA patterns can eb defined early in the disease. This is important considering the emphasis on early diagnosis that will identify milder cases of SSc and so predictors of rapid progression are especially valuable.