Plasma endogenous enkephalin levels in early systemic sclerosis: clinical and laboratory associations

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General Clinical Research Centers of participating universities and written informed consent was obtained from all subjects before study entry. The GENISOS samples were patients with early SSc who met American College of Rheumatology diagnostic criteria within 5 years of diagnosis, or onset of the first symptom other than Raynaud’s phenomenon, detailed elsewhere (9, 10). Patients were diagnosed with systemic sclerosis (SSc) if they met the ACR proposed classification criteria (10). Assignment of diffuse cutaneous SSc was further based on the proximal distribution of skin involvement, that is, thickened skin involvement detected above the elbows or knees, exclusive of involvement of the face (11). Nailfold capillaroscopy was not performed as part of the GENISOS study. The patients were enrolled throughout the year. The catchment area of the three participating university centres (UTMB, UTH-HSC, and UTHSC-SA) roughly encompasses a 500-mile area in Southeastern Texas, with a latitude range of 29.3–29.75 degrees. The overall daily temperatures range from 14–33°C (12). All clinical visits were performed in climate controlled buildings, usually 23–26°C for patient comfort. Plasma samples (n=116) were aliquoted and stored at -80°C until HPLC analysis for enkephalin determinations was performed by blinded investigators. For HPLC analysis, thawed filtered samples were subjected to reverse phase HPLC with coulometric electrochemical detection (ESA product 5010A) for met-enkephalin and leu-enkephalin (13). For statistical analyses, nonparametric tests were performed to determine if our sample size was adequate to attain significance in the study. Multiple linear regression analyses were conducted to investigate persistently significant differences after controlling the effects of age, race and gender. Data are presented as mean ± standard deviation unless otherwise specified. A p-value <0.05 was considered significant. Power analysis was performed to determine if the sample size was appropriate to attain significance by multiple regression analysis (14).

### Results

The racial/ethnic composition of the SSc group was as follows: Caucasian, 47%; African American, 21%; Hispanic, 28% and others, 4%. The average age for SSc patients was 50.1±12.3 years. The percentage of females was 86%. The percentage with diffuse cutaneous involvement was 59%. The percentage who used tobacco (smoked cigarettes) was 17%. The average total skin score was 15.1±12.4.

Results of univariate analyses for plasma enkephalin levels, autoantibodies and clinical variables are shown in Table I. The plasma met-enkephalin levels were significantly lower in SSc patients who were seropositive for TOPO-I antibodies, compared to those who were seronegative (6.1±8.3 vs. 14.9±22.8 μg/ml, respectively, p=0.02). No significant correlations with plasma met- and leu-enkephalin levels were seen for samples seropositive for the following autoantibodies: antinuclear, anti-centromere, anti ribonucleotidease, Th/TO, polymerase I, polymerase II, polymerase III or anti-fibrillarin. Mean plasma leu-enkephalin levels were significantly higher in the presence of digital pulp loss, compared to the patients without pulp loss, as shown (97.0±134 vs. 65.1±100 μg/ml, respectively, p=0.04). Mean plasma leu-enkephalin levels were significantly lower in the presence of myositis, as shown (97.0±134 vs. 65.1±100 μg/ml, respectively, p=0.047). Only five patients had myositis at the time of the study visit.

### Table I. Association of plasma enkephalin levels to clinical variables.

<table>
<thead>
<tr>
<th>Scleroderma Clinical Profile</th>
<th>Variable (n.)</th>
<th>Met-Enk, (μg/ml)</th>
<th>p-value</th>
<th>Leu-Enk, (μg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum TOPO-I</td>
<td>Yes (23)</td>
<td>6.1±8.3</td>
<td>0.02</td>
<td>80.1±99.1</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>No (93)</td>
<td>14.9±22.8</td>
<td></td>
<td>74.1±116.0</td>
<td></td>
</tr>
<tr>
<td>Loss of digital pulp</td>
<td>Yes (37)</td>
<td>8.5±13.4</td>
<td>0.10</td>
<td>97.0±134</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>No (79)</td>
<td>14.8±23.3</td>
<td></td>
<td>65.1±100</td>
<td></td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>Yes (109)</td>
<td>12.8±21.4</td>
<td>0.07</td>
<td>78.2±114.8</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>No (7)</td>
<td>16.6±5.9</td>
<td></td>
<td>25.9±29.2</td>
<td></td>
</tr>
<tr>
<td>Tendon friction rubs</td>
<td>Yes (10)</td>
<td>3.5±3.2</td>
<td>0.06</td>
<td>128.3±236.4</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>No (106)</td>
<td>14.0±21.7</td>
<td></td>
<td>70.0±92.0</td>
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</tr>
<tr>
<td>Myositis</td>
<td>Yes (5)</td>
<td>9.9±14.6</td>
<td>0.74</td>
<td>14.3±11.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>No (111)</td>
<td>13.2±21.2</td>
<td></td>
<td>77.7±113</td>
<td></td>
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<tr>
<td>Telangiectasias</td>
<td>Yes (27)</td>
<td>12.1±16.4</td>
<td>0.47</td>
<td>44.6±58.9</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>No (89)</td>
<td>13.3±22.1</td>
<td></td>
<td>85.0±123.2</td>
<td></td>
</tr>
<tr>
<td>Diffuse cutaneous SSc</td>
<td>Yes (65)</td>
<td>12.5±16.4</td>
<td>1.00</td>
<td>84.4±128.1</td>
<td>0.72</td>
</tr>
<tr>
<td>Limited cutaneous SSc</td>
<td>Yes (51)</td>
<td>13.8±26.0</td>
<td></td>
<td>64.6±90.6</td>
<td></td>
</tr>
</tbody>
</table>

Association of plasma enkephalin levels and clinical parameters in early SSc. Plasma samples and clinical data were obtained from the baseline study visit of the GENISOS database. Plasma Met-enkephalin and leu-enkephalin levels were expressed as mean values ± standard deviation. The presence (yes, “present today”) or absence (no) of the parameter at the time of the study visit is noted and the (number) represents the number of patients in the database. The p-value demonstrates the level of significance from univariate analysis. The number of samples with the present variable (“yes”) or absence (“no”) for a clinical or laboratory variable are shown in parentheses () for those variable that were present at the time of the study visit.
Mean plasma met-enkephalin levels were modestly decreased in patients who reported Raynaud’s phenomenon (RP), compared to those who did not (12.8±21.4 vs. 16.6±5.9 ug/ml, respectively, p=0.07). The SSc patients with RP had increased plasma leu-enkephalin levels compared to absence of telangiectasias (44.6±58.9 vs. 25.9±29.2 mg/ml, p=0.01). SSc patients with RP (n=109) compared to those with no RP (n=7) at the time of study visit had higher frequencies of sclerodactyly (96% vs. 71%, p=0.042) and digital pits/ulcers (66% vs. 29%, p=0.097). Mean plasma met-enkephalin levels were also modestly decreased in the presence compared to absence of tendon friction rubs (3.5±3.2 vs. 14.0±21.7, p=0.06). Mean plasma leu-enkephalin levels were decreased in the presence compared to absence of telangiectasias (44.6±58.9 vs. 85.0±123.2, p=0.09). Minimal differences in mean plasma met-enkephalin levels were noted in dSSc patients compared to lSSc patients (12.5±16.4 vs. 13.8±26.0, p=1.0). Mean plasma leu-enkephalin levels were not significantly higher in dSSc patients compared to lSSc patients (84.4±128.1 vs. 64.6±90.6, p=0.72). No associations were noted between plasma enkephalin levels and other digital manifestations (total skin score, digital ulcers or digital gangrene), systemic manifestations, scores from the Scleroderma-HAQ, SF-36 surveys, or medications. The modified MSS (composite scores or scores from nine individual organ systems) did not associate with the plasma endogenous enkephalin levels in early SSc.

Significant effects noted by univariate analysis by t-test were not sustained after controlling for age, gender and race by multiple linear regression. Power analysis determined our sample size was not large enough to achieve 80% power at a 5% significance level for the association of TOPO-I, digital pulp loss or myositis with plasma enkephalin levels, (needed ≥190 patients, 14).

Discussion
These data are consistent with our hypotheses that decreased plasma met-enkephalin levels are vasogenic mediators in SSc, and will associate with clinical manifestations of SSc-related vascular and fibrogenic injuries. Plasma met-enkephalin levels were modestly to significantly lower in patients seropositive for TOPO-I and in patients with loss of digital pulp, RP, tendon friction rubs, myositis, telangiectasias and diffuse cutaneous involvement. Plasma leu-enkephalin levels were higher in patients seropositive for TOPO-I and with loss of digital pulp, RP and tendon friction rubs. We predict that met-enkephalin levels will be inversely associated with vasogenic and fibrogenic activity in early SSc. It will be important to study plasma enkephalin fluctuations in larger group numbers over time in the context of SSc disease activity and progression.

Recent studies highlight an emerging appreciation for endogenous opioid and opioid receptor interactions in vaso-dilatory, anti-apoptotic and anti-inflammatory functions (6-8). Elevated endothelin-1 levels reported in SSc may mediate vasoconstriction in peripheral vessels, in part, by modulation of met-enkephalin. In a percussive brain injury model, endothelin receptor activation decreased met-enkephalin mediated cerebral blood flow. Application of an endothelin-1 antagonist restored endogenous opioid and opioid receptor interactions (8).

TOPO-1 autoantibody seropositivity is associated with skin and vascular changes (13). TOPO-1 autoantibodies have been reported to increase endothelial apoptosis, pro-fibrogenic activation and digital contractures (5, 15, 16). TOPO-1 immune complexes may
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increase endothelin-1 and subsequently decrease met-enkephalin levels, to trigger or enhance cellular apoptosis in endothelial injury. SSc specific antibodies (i.e. anti-centromere antibodies or TOPO-1) may also contribute to peripheral tissue injury by autonomic dysfunction (17).

Enkephalin levels may also directly impact fibrogenic processes. The association of higher plasma leukocyte enkephalin levels with loss of digital pulp in SSc patients supports their potential relevance in subsequent tissue remodeling from increased fibrogenic activity. Reported physiologic interactions of cytokines such as transforming growth factor-beta expression and opioid agonists possibly contribute to wound healing (18). Indirectly, lower met-enkephalin levels have been demonstrated to promote apoptosis via the cellular caspase-3 pathway in cultured cells (7). Serum autoantibodies from SSc patients are reported to increase apoptosis of cultured dermal endothelial cells by a similar mechanism (15). Fig. 1 illustrates selected proposed cellular targets of decreased endogenous enkephalin expression in vasogenic and fibrogenic injury. Below are selected studies that support a proposed physiologic relevance of plasma endogenous opioid levels in SSc and their potential as therapeutic targets.

1. Circulating endogenous enkephalin levels may have modest physiologic influences under normal conditions, but become prominent regulators of vascular tone during episodes of injury or increased stress, such as persistent tissue hypoxia (8).

2. Mu opioid receptor agonists demonstrate vasconstrictive properties at low and vasodilatory properties at high concentrations in cardiovascular models (19).

3. Supplementation with other neurotransmitter agonists has induced vasodilation in secondary Raynaud’s and animal models, supporting a potential physiologic impact of plasma mediators in peripheral tissue processes (3, 20).

4. Two-fold increased plasma β-endorphin levels in psoriatic patients (compared to normal controls) were attributed to reduced capsaicin-induced neurogenic inflammatory responses in psoriatic skin (21), supporting a physiologic (and potentially pathologic) consequence of plasma opioid levels.

5. A recent study has demonstrated that treatment with α-melanocyte stimulating hormone (a gene product of the POMC gene), suppressed skin fibrosis in a bleomycin mouse model of SSc (22). This supports a role of opioid related gene products in fibrotic processes.

6. Digital transcutaneous electrical nerve stimulation (TENS), which increases opioid peptide levels (reviewed in 23), promotes peripheral vasodilation, with improved blood flow to the digits (24). Electrical neural stimulation can promote selective release of neurotransmitters, including opioid peptides (25).

Plasma enkephalins and tissue opioid receptors may provide novel or additive therapeutic targets for restoring or increasing tissue oxygenation in the inflammatory and vasculopathic processes of anoxic injury. Studies with larger patient enrollment are needed to determine if plasma or tissue enkephalin levels are physiologically relevant and which mechanisms of action are most pertinent to scleroderma pathogenesis. Future studies with capillaroscopy, which is noninvasive, and enkephalin levels would be desirable as significant associations of enkephalin levels with parameters of vascular integrity would be predicted (26, 27). Ultimately, if plasma enkephalins are depleted by repeated episodes of vasospasm, SSc patients subsets with increased risk of hypoxic injury may need additional therapies to minimise vascular or fibrotic injury (28).

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References


12. Climate and topographical records from the National Oceanic Atmospheric Administration (NOAA), www.nws.noaa.gov/}


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