Connective Tissue Growth Factor Is Associated With a Stable Atherosclerotic Plaque Phenotype and Is Involved in Plaque Stabilization After Stroke

Jan Willem Leeuwis, MD; Tri Q. Nguyen, MD, PhD; Maarten G.J. Theunissen, MD; Wouter Peeters, MD, PhD; Roel Goldschmeding, MD, PhD; Gerard Pasterkamp, MD, PhD; Aryan Vink, MD, PhD

Background and Purpose—Carotid plaques remodel toward a more stable phenotype after stroke, but not after TIA. Connective tissue growth factor (CTGF) is involved in extracellular matrix production and is expressed in atherosclerotic plaques. We studied the role of CTGF in plaque remodeling after stroke and TIA.

Methods—Atherosclerotic plaques from carotid endarterectomy of asymptomatic patients (n=16) and patients who experienced stroke (n=15) or TIA (n=33) were analyzed for CTGF levels, markers of plaque stability (collagen, smooth muscle cells, macrophage content, and lipid core), and levels of matrix metalloproteinase (MMP)-8, MMP-9, IL-4, IL-5, and IL-10.

Results—CTGF levels were higher in stroke patients compared to TIA patients. Plaques with a high level of CTGF revealed more collagen and smooth muscle cell content, whereas macrophage content and lipid core size were not different. The amount of CTGF was negatively associated with MMP-8 and MMP-9 activity and showed a positive correlation with the anti-inflammatory cytokines IL-4, IL-5, and IL-10.

Conclusions—CTGF levels are associated with a more stable plaque phenotype. CTGF is increased in plaques after stroke compared to TIA, suggesting a role for CTGF in plaque stabilization after stroke. (Stroke. 2010;41:2979-2981.)

Key Words: atherosclerosis ■ carotid endarterectomy ■ plaque stability

Over the past years it has become increasingly clear that atherosclerosis is a dynamic process. 1–3 Vulnerable carotid plaques, either by plaque erosion or plaque rupture, are prone to induce thromboembolic stroke or TIA. 4 After stroke, atherosclerotic plaques remodel over time toward a more stable plaque, whereas after TIA plaques remain unchanged. 2,3 Identification of plaque constituents influencing this remodeling would enable risk assessment for future events and identify targets for medical therapy.

Connective tissue growth factor (CTGF, also known as CCN2 [Cyr61/CTGF/NOV]) is a profibrotic growth factor that induces proliferation of vascular smooth muscle cells and accumulation of extracellular matrix by inducing collagen production and inhibiting matrix breakdown. 5 CTGF expression is elevated in atherosclerosis compared to normal arteries. 6 We studied the role of CTGF in plaque remodeling after stroke.

Materials and Methods

The Athero-Express study is a vascular biobank study. 7 Sixty-four patients undergoing carotid endarterectomy were included in this study. Written informed consent was obtained from all patients.

The excised plaques were processed and examined as described previously. 2 Collagen (Sirius Red), macrophages (CD68), and smooth muscle cells (α-actin) were rated as no/minor or moderate/heavy. The size of the extracellular lipid core was scored as <10%, 10% to 40%, or >40% of total plaque size. Paraffin sections were stained for CTGF using a mouse anti-human CTGF monoclonal antibody (FibroGen).

Isolated total protein was used for activity measurements (matrix metalloproteinase [MMP]-8 and MMP-9, Biotrak assays; Amersham Biosciences) and fluorescent bead immunoassay (IL-4, IL-5, and IL-10; Bendermed Systems) as described previously. 2 Western blot analysis of CTGF was performed using goat anti-human CTGF monoclonal antibody (sc-14939; Santa Cruz Biotechnologies).

For statistical analysis, differences were calculated using the 2 test, Mann-Whitney test, or Kruskal-Wallis test with Dunn post hoc multiple comparison. Associations were tested using Spearman rank correlation.

Results

Patients presented with stroke (n=15), TIA (n=33), or were asymptomatic (n=16). No differences in CTGF levels were found for known cardiovascular risks (Table). However, higher CTGF levels were detected in plaques from stroke
patients compared to TIA patients (respectively, 278 vs 195 arbitrary units; P<0.05; Figure 1A,B). Immunohistochemical staining revealed CTGF expression in (myo)fibroblasts, surrounding microvessels, in fibrotic areas, and in between inflammatory regions of the plaque (Figure 1C–F).

To investigate a possible role for CTGF in plaque stabilization, plaques were divided in two groups based on the median CTGF level (175 arbitrary units). Plaques with high CTGF levels revealed more collagen (P=0.026) and smooth muscle cells (P=0.031; Figure 2A,B). No differences were found for macrophages (P=0.451) and size of lipid core (P=0.520; Figure 2B,C). Because CTGF staining was mainly observed in noninflammatory areas, we investigated the correlation of CTGF with anti-inflammatory markers IL-4, IL-5, and IL-10, which were positively correlated with the amount of CTGF (IL-4, ρ=0.611, P<0.001; IL-5, ρ=0.306, P=0.02; IL-10, ρ=0.468, P<0.001; Figure 2E–G). The activity of the matrix-degrading enzymes MMP-8 and MMP-9 was negatively associated with CTGF levels (MMP-8, ρ=−0.407, P=0.001; MMP-9, ρ=−0.267, P=0.036; Figure 2H,I).

**Discussion**

The results of this study show that carotid plaques from stroke patients have higher CTGF levels compared to plaques from TIA patients. In addition, we show that CTGF is associated with stable plaque characteristics.

Atherosclerosis is considered a dynamic process in which plaque stabilization and destabilization alternate depending on local and systemic factors. We and others have recently demonstrated that plaques become more stable over time after stroke, whereas after a TIA plaque phenotype does not change. Our results suggest that CTGF is upregulated after stroke.

CTGF is a profibrotic factor involved in smooth muscle cell proliferation and extracellular matrix production. We observed increased smooth muscle cell and collagen content in plaques with high CTGF levels, suggesting that CTGF is involved in plaque stabilization.

We report a negative association between CTGF and the matrix degradation proteins MMP-8 and MMP-9, which are upregulated in unstable atherosclerotic plaques. In a mouse model, we have recently demonstrated that CTGF inhibits MMP activity. Our observation suggests a role for CTGF in the inhibition of plaque MMP levels.

In conclusion, the results of this cross-sectional study suggest CTGF may have a role in changing plaque compo-

**Table. Patient Characteristics in Relation to Plaque CTGF Levels**

<table>
<thead>
<tr>
<th>CTGF Levels (Arbitrary Units) and Median (Interquartile Range)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong>&lt;br&gt;67 (43–83)</td>
<td>0.334</td>
</tr>
<tr>
<td><strong>Gender</strong>&lt;br&gt;Male&lt;br&gt;47/64 73.4 162 (113–278)</td>
<td>0.539</td>
</tr>
<tr>
<td>Female&lt;br&gt;17/64 26.6 214 (116–373)</td>
<td>0.520</td>
</tr>
<tr>
<td><strong>Diabetes</strong>&lt;br&gt;Yes&lt;br&gt;11/64 82.8 174 (105–533)</td>
<td>0.051</td>
</tr>
<tr>
<td>No&lt;br&gt;53/64 17.2 177 (114–282)</td>
<td>0.229</td>
</tr>
<tr>
<td><strong>Smoker</strong>&lt;br&gt;Yes&lt;br&gt;12/64 81.3 147 (102–251)</td>
<td>0.421</td>
</tr>
<tr>
<td>No&lt;br&gt;52/64 18.8 182 (117–340)</td>
<td>0.297</td>
</tr>
<tr>
<td><strong>Hypertension</strong>&lt;br&gt;Yes&lt;br&gt;40/64 62.5 207 (116–289)</td>
<td>0.453</td>
</tr>
<tr>
<td>No&lt;br&gt;24/64 37.5 147 (103–308)</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Hypercholesterolemia</strong>&lt;br&gt;Yes&lt;br&gt;36/64 56.3 183 (103–289)</td>
<td>0.033</td>
</tr>
<tr>
<td>No&lt;br&gt;28/64 43.8 156 (127–324)</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>BMI</strong>&lt;br&gt;≤25 kg/m²&lt;br&gt;22/61 36.1 199 (118–301)</td>
<td>0.797</td>
</tr>
<tr>
<td>&gt;25 kg/m²&lt;br&gt;39/61 63.9 150 (107–290)</td>
<td>0.542</td>
</tr>
<tr>
<td><strong>Event-operation period</strong>&lt;br&gt;≤60 days&lt;br&gt;11/44 25.0 214 (143–275)</td>
<td>0.453</td>
</tr>
<tr>
<td>&gt;60 days&lt;br&gt;33/44 75.0 150 (107–289)</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>Clinical presentation</strong>&lt;br&gt;Asymptomatic&lt;br&gt;16/64 25.0 191 (113–348)</td>
<td>0.033</td>
</tr>
<tr>
<td>TIA&lt;br&gt;33/64 51.6 146 (102–232)</td>
<td>0.042</td>
</tr>
<tr>
<td>Stroke&lt;br&gt;15/64 23.4 278 (150–416)</td>
<td>0.051</td>
</tr>
</tbody>
</table>

CTGF indicates connective tissue growth factor.

**Notes:**

To investigate a possible role for CTGF in plaque stabilization, plaques were divided in two groups based on the median CTGF level (175 arbitrary units). Plaques with high CTGF levels revealed more collagen (P=0.026) and smooth muscle cells (P=0.031; Figure 2A,B). No differences were found for macrophages (P=0.451) and size of lipid core (P=0.520; Figure 2B,C). Because CTGF staining was mainly observed in noninflammatory areas, we investigated the correlation of CTGF with anti-inflammatory markers IL-4, IL-5, and IL-10, which were positively correlated with the amount of CTGF (IL-4, ρ=0.611, P<0.001; IL-5, ρ=0.306, P=0.02; IL-10, ρ=0.468, P<0.001; Figure 2E–G). The activity of the matrix-degrading enzymes MMP-8 and MMP-9 was negatively associated with CTGF levels (MMP-8, ρ=−0.407, P=0.001; MMP-9, ρ=−0.267, P=0.036; Figure 2H,I).

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**Figure 1.** A, Connective tissue growth factor (CTGF) expression is higher in plaques from stroke patients compared to TIA patients. B, Western blot of CTGF in a stroke patient and a TIA patient. Immunohistochemical staining revealed CTGF-positive cells (arrows) in the cap and shoulder region (C), in myofibroblasts in fibrotic area (D), in between inflammatory regions near the lipid core (E), and surrounding microvessels (F).
sition toward a stable plaque after stroke. Further longitudinal studies are needed to confirm these results.

Acknowledgments
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Disclosures
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References
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