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Dimitrijevic, Ivan; Malmsjö, Malin; Andersson, Christina; Rissler, Pehr; Edvinsson, Lars

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Corticosteroids are the only proven treatment for giant cell arteritis. This study shows upregulated angiotensin II type 1 receptors in both smooth muscle cells and inflammatory cells in the temporal arteries from patients with GCA.
Increased angiotensin II type 1 receptor expression in temporal arteries from patients with giant cell arteritis

Ivan Dimitrijevic M.D., a,b Malin Malmsjö M.D., PhD, c Christina Andersson M.T., c Pehr Rissler M.D., a Lars Edvinsson M.D., PhD.

aDepartment of Emergency Medicine, bDepartment of Ophthalmology and cDepartment of Pathology, Clinical Sciences, Lund, Lund University, Sweden

Short title: Angiotensin II and giant cell arteritis

Corresponding author:

Ivan Dimitrijevic

Division of Experimental Vascular Research

BMC A13

SE-221 84 Lund

Sweden

Telephone number +46 46-176 749

Fax number +46 46-222 0616

e-mail: ivan.dimitrijevic@med.lu.se

KEY WORDS: AT1 receptor; AT2 receptor; immunohistochemistry; temporal artery, giant cell arteritis, vascular

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Purpose
Currently, giant cell arteritis (GCA) is primarily treated with corticosteroids or immunomodulating agents, but there is interest in identifying other non-corticosteroid alternatives. Similarities exist in the injury pathways between GCA and atherosclerosis. Angiotensin II is a vasoactive peptide involved in vessel inflammation during atherosclerosis, and angiotensin II receptor inhibitors are effective in preventing atherosclerosis. The present study was performed to elucidate the role of angiotensin type 1 (AT₁) and type 2 (AT₂) receptors in GCA.

Participants
Ten patients with GCA and ten control patients, who were clinically suspected of having GCA but were diagnosed as not having GCA, were included.

Methods
Immunohistochemistry, using anti-AT₁ and anti-AT₂ antibodies, was performed on formalin-fixed and paraffin-embedded temporal arteries.

Main outcome measures
AT₁ and AT₂ receptor immunostaining intensity was quantified.

Results
Hematoxylin-eosin-stained sections of temporal arteries from patients with GCA showed intimal hyperplasia, internal elastic lamina degeneration and band-shaped infiltrates of inflammatory cells, including lymphocytes, histocytes, and multinucleated giant cells. AT₁
receptor staining was primarily observed in the medial layer of the temporal arteries, and was higher in the patients with GCA than in the control patients. This was a result of increased AT$_1$ receptor immunostaining of both vascular smooth muscle cells and of infiltrating inflammatory cells. Only faint immunostaining was seen for AT$_2$ receptors, primarily in the endothelial cells, and to a lesser extent on the smooth muscle cells. Immunostaining with antibodies for the AT$_2$ receptor was similar in the patients with GCA and in controls.

**Conclusions**

These results suggest that AT$_1$ receptors play a role in the development of GCA. Inhibition of the angiotensin system may thus provide a non-corticosteroid alternative for the treatment of GCA.
Introduction

Giant cell arteritis (GCA) is a granulomatous form of vasculitis involving large and medium-sized vessels. The extracranial branches of the aorta are the main targets, and the major source of diagnostic biopsies is the temporal artery. The clinical manifestations of GCA are due to end organ ischemia and include blindness, jaw claudication, headaches, stroke and aortic arch syndrome. These symptoms are often accompanied by signs of systemic inflammation, such as malaise, anorexia, fever and myalgia. Excessive amounts of acute phase proteins are produced and the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and interleukin-6 levels are elevated.

GCA is a form of panarteritis with transmural inflammation, involving the media, intima and adventitia of the affected arteries. Active disease is characterized by a nodular granulomatous reaction that is centered around a fragmented internal elastic lamina with inflammatory cells in the intima or media. The typical lesions are formed by interferon-γ-producing CD4+ T cells and macrophages. Thrombosis may be present, although ischemic symptoms are usually the result of lumen narrowing resulting from mural thickening.

Currently, giant cell arteritis (GCA) is primarily treated with corticosteroids or immunomodulating agents. However, there is interest in identifying other non-corticosteroid alternatives because of the numerous complications associated with long-term corticosteroid treatment. The complications appear to be dose related, and non-corticosteroid medication may therefore be beneficial. Studies have been carried out on methotrexate, anti-TNF-α agents, and others, such as azathioprine and cyclophosphamide, but none of these has yet
been recommended for GCA. Modulating the inflammatory response by inhibiting the renin-angiotensin system may be an alternative approach for the treatment of GCA.

Angiotensin II receptor inhibitors are effective in preventing atherosclerosis. GCA and atherosclerosis have similar injury pathways, both of which involve vessel inflammation. Angiotensin II is a vasoactive peptide that is known to play a role in vessel inflammation in atherosclerosis. Angiotensin II modulates the immune response via the production of NF-κB in the cytoplasm of mature phagocytes, resulting in the transcription of inflammatory cytokines and chemokines, including IL-1beta, IL-6, IFN-gamma and TNF-alpha, and IL-8, MCP-1 and CAM-1, respectively. Angiotensin-converting enzyme inhibitors reduce the expression of monocyte chemo-attractant protein-1 and concomitant macrophage plaque infiltration in animal models of atherosclerosis. In patients with carotid artery disease, the angiotensin receptor blocker irbersartan inhibits metalloproteinase activity, as well as T-cell and macrophage infiltration in the vascular wall.

To our knowledge, no study has yet been performed to elucidate the possible role of angiotensin II receptors in GCA in humans. In the present study, angiotensin type 1 (AT₁) and type 2 (AT₂) receptor expression was investigated in temporal arteries, using immunohistochemistry. The immunostaining intensity in temporal arteries from patients with GCA was compared to that in arteries from control patients. The latter were clinically suspected of having GCA, but this diagnosis was ruled out.
MATERIALS AND METHODS

Tissue collection

Temporal artery vessel biopsy specimens from 20 patients were retrieved from the Department of Pathology, Lund University, Sweden, where they had been routinely examined for histopathology. All patients’ symptoms and background characteristics are listed in Table 1. The original pathology reports were reviewed to verify the diagnosis GCA. The diagnosis was based on the American College of Rheumatology 1990 criteria for the classification of GCA\textsuperscript{13}. The absence of inflammatory lesions in a sample led to the categorization of the sample as negative. Temporal arteries from ten patients with GCA were compared to arteries from ten patients who were clinically suspected of having GCA, but the diagnosis had been ruled out (control patients). The histopathological findings in the temporal arteries from all 20 patients are listed in Table 2.

Ethics

The samples were handled in accordance with permission obtained from the Lund University Human Ethics Committee (LU 818-01), and the study conformed to the principles outlined in the Declaration of Helsinki.

Immunohistochemistry

Four-μm sections were cut from formalin-fixed, paraffin-embedded blocks and dried at 60 °C for one hour. After dewaxing and rehydration, the sections were treated with 10 mM citrate buffer, pH 6.0, in a microwave oven for 15 min for antigen retrieval. Immunohistochemical staining with rabbit polyclonal antibody AT\textsubscript{1} receptor (sc-1173) and AT\textsubscript{2} receptor (sc-9034, both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) diluted 1:50, anti CD68 (Dako
M876) diluted 1:200, and anti CD45 (Dako M701) diluted 1:600, smooth muscle actin
diluted 1:500 (Dako M0851) and CD31 diluted 1:50 (Dako M823) was performed in an
automated immunostainer, TechMate 500 (Ventana Biotek, Tuscan, AZ, USA), using the
biotin-streptavidin-peroxidase method with diaminobenzidine as the chromogen (Dako
REAL™ Detection System, Peroxidase/DAB+, Rabbit/Mouse). Mayer’s hematoxylin was
used for counterstaining. Primary antibodies were omitted for negative control. Details and
antibody specificity are given on the company’s homepage. The samples were examined with
a microscope (Olympus BX60, Tokyo, Japan) and photographed using a digital camera
(Pixera Pro 600ES, Los Gatos, CA, USA).

Calculations and statistics

Calculations were performed on the results obtained from temporal arteries from 10 patients
with GCA and 10 control patients. The immunofluorescence intensity was measured using
ImageJ (http://rsb.info.nih.gov/ij/). Measurements were performed on three sections, at four
different regions in each sample, and the mean values were calculated. Statistical analysis was
performed using Student’s t-test. Significance was defined as \( P<0.05 \) (*), \( P<0.01 \) (**) and
\( P<0.001 \) (***) Values are presented as means ± standard deviation (SD).
Results

Hematoxylin-eosin staining

Hematoxylin-eosin-stained sections of temporal arteries from patients with GCA showed intimal hyperplasia with reduction of the lumen, internal elastic lamina degeneration and band-shaped infiltrates of inflammatory cells, including lymphocytes, histocytes, and multinucleated giant cells (Table 2 and Figure 1). Immunostaining for CD68 demonstrated strong labeling in the area of inflammatory cell infiltrates. Immunostaining for CD45 was also seen, but was not as prominent. In the more severe cases of GCA, transmural inflammatory infiltrates and necrosis were seen in portions of the vascular wall. The control patients were clinically suspected cases of GCA, in which the diagnosis of GCA had been ruled out. The temporal arteries from these control patients had intimal hyperplasia and sub-intimal fibrosis and focal loss of internal elastic lamina, suggestive of atherosclerotic disease.

AT₁ receptor immunostaining

Immunostaining for AT₁ receptors was observed in the smooth muscle cell layer and in the endothelial cell layer (Figure 2) in temporal arteries from control patients. In the smooth muscle cell layer of the temporal arteries from patients with GCA there was a 0.55 fold increase in the immunostaining intensity of AT₁ receptor in the smooth muscle layer compared to control patients (40 ± 22 in controls and 73 ± 28 in patients with GCA, p<0.05). No changes in immunostaining of AT₁ receptors were detected in the endothelial cell layer in temporal arteries from controls or GCA patients (56 ± 20 in controls and 60 ± 19 in patients with GCA, p=n.s.). In the temporal arteries from the patients with GCA, AT₁ receptor staining was observed in the lymphocytes, histocytes, multinucleated giant cells, and smooth muscle cells, as well as in the neointima (Figure 2).
**AT₂ receptor immunostaining**

Only faint immunostaining was seen for AT₂ receptors, primarily in the endothelial cell layer, and to a lesser extent in the smooth muscle cell layer. No difference was observed in the AT₂ receptor immunostaining intensity between the temporal arteries of patients with GCA and controls in the smooth muscle cell layer (36 ± 18 in controls and 41 ± 22 in patients with GCA, p=n.s.) or in the endothelial cell layer (49 ± 17 in controls and 47 ± 20 in patients with GCA, p=n.s., Figure 3).

Counterstaining with alpha-gamma actin and CD31 verified the presence of AT₁ and AT₂ receptors in smooth muscle and endothelial cells, respectively.
Discussion

Major findings
The objective of this study was to investigate the possible roles of AT₁ and AT₂ receptors in GCA. Temporal arteries from patients diagnosed with GCA were compared to those from control patients that were clinically suspected cases but the diagnosis of giant cell arteritis had been ruled out. The major finding of the present study was increased immunostaining intensities for AT₁ receptors in both smooth muscle cells and inflammatory cells in the temporal arteries from patients with GCA.

GCA diagnosis
The patients included in the present study were diagnosed as having GCA according to the American College of Rheumatology 1990 criteria for the classification of giant cell arteritis, which have a sensitivity of 94% and a specificity of 91%. The criteria were initially developed as a research tool, and the presence of three or more of the following criteria are required for the diagnosis of GCA: age of onset greater than 50 years, onset of new headache, temporal artery abnormality (tender or reduced pulsation), elevated ESR, defined as 50 mm/h, and abnormal arterial biopsy. Table 1 gives the characteristics of the patients’ clinical presentation.

Hematoxylin-eosin-stained sections of temporal arteries demonstrated features that are typical of giant cell arteritis, including intimal hyperplasia with reduction of the lumen, internal elastic lamina degeneration and band-shaped infiltrates of inflammatory cells, including lymphocytes, histocytes, and multinucleated giant cells. In the more severe cases of GCA,
transmural inflammatory infiltrates and necrosis were seen in parts of the vascular wall. Table 2 provides a detailed presentation of the vascular wall lesions in study patients with GCA.

Angiotensin II receptors in the vascular wall

Two angiotensin II receptors have been identified and cloned in man: the AT₁ and AT₂ receptors.¹⁵, ¹⁶ AT₁ and AT₂ receptors are distributed heterogeneously in human tissues and blood vessels.¹⁷ In the present study, immunostaining for AT₁ receptors was seen on the smooth muscle cells of the media in the temporal arteries from the control patients. Only faint immunostaining was seen for AT₂ receptors, primarily in the endothelial cell layer, and to a lesser extent in the smooth muscle cell layer. This is in accordance with previous findings of AT₁ receptors on smooth muscle cells, and AT₂ receptors on endothelial cells.¹⁸-²¹ Immunostaining for AT₂ on the smooth muscle cells of arteries has also been reported.²²

In the present study, the levels of AT₁ receptors on smooth muscle cells were higher in the temporal arteries from the patients with GCA than in the temporal arteries from the control patients. Angiotensin II is a potent vasoconstrictor and mitogen of vascular smooth muscle cells. The stimulation of AT₁ receptors is known to result in progression of atherosclerotic lesions, inflammation and plaque rupture.²³, ²⁴ The upregulation of AT₁ receptors in GCA could make the arteries prone to tonic contraction and spasm, resulting in the progression of vascular wall inflammation.

Angiotensin II receptors and vascular wall inflammation

High AT₁ receptor immunostaining intensity in arteries from patients with GCA was not only due to increased protein expression on the smooth muscle cells, but also to high levels of AT₁ receptor expression on inflammatory cells, including lymphocytes and macrophages
infiltrating the vascular lesions. AT₁ receptors have previously been demonstrated on both lymphocytes and macrophages.²⁵-²⁷ Angiotensin II affects human T-lymphocyte function via AT₁ receptor activation, which results in increased production of pro-inflammatory cytokines such as interferon-γ and TNF-α.²⁸ ²⁹ The intense expression of the AT₁ receptor in inflammatory cells in the GCA vascular lesion may contribute to the progression of the disease. We suggest that inhibiting the angiotensin system may offer a new means of pharmacologically modulating the progression of GCA. Indeed, inhibitors of AT₁ receptors have been shown to modify the progression of atherosclerosis and stabilize atheromatous plaques.¹¹, ¹² Atherosclerosis and GCA are both vascular diseases in which inflammation is a common entity.

Conclusions

The present study demonstrates high levels of AT₁ receptor expression in both the vascular smooth muscle cells and inflammatory cells in the temporal arteries of patients with GCA. Increased activity in the angiotensin II system may play a role in the progression of inflammation in GCA. We suggest that inhibition of the renin-angiotensin system may provide a non-corticosteroid alternative for the treatment of GCA inflammation.
References


Table 1 Background characteristics of all patients included in the study.

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<thead>
<tr>
<th></th>
<th>Controls</th>
<th>GCA</th>
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<tbody>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
</tr>
<tr>
<td>Median age (range) (years)</td>
<td>70 (54-92)</td>
<td>74 (66-88)</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>6 / 4</td>
<td>4 / 6</td>
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<tr>
<td>Headache</td>
<td>2</td>
<td>7</td>
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<tr>
<td>Jaw claudication</td>
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<tr>
<td>Polymyalgia rheumatica</td>
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<td>1</td>
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<tr>
<td>Temporal artery pain</td>
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<td>9</td>
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<tr>
<td>Abnormal temporal artery</td>
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<td></td>
</tr>
<tr>
<td>on palpation</td>
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<td>4</td>
</tr>
<tr>
<td>Any visual symptom</td>
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<td>6</td>
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<tr>
<td>Diplopia</td>
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<td>0</td>
</tr>
<tr>
<td>Scalp tenderness</td>
<td>3</td>
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<td>Anorexia</td>
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<tr>
<td>Fever</td>
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<td>4</td>
</tr>
<tr>
<td>Myalgia</td>
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</tr>
<tr>
<td>Arthralgia</td>
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<td>ESR &lt; 50 mm/h</td>
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<tr>
<td>ESR 50-100 mm/h</td>
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<td>10</td>
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<tr>
<td>Condition</td>
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<td>Group 2</td>
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<td>---------</td>
<td>---------</td>
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<tr>
<td>ESR &gt; 100 mm/h</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>29 ± 26</td>
<td>137 ± 37</td>
</tr>
<tr>
<td>Anemia</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Abnormal arterial biopsy</td>
<td>0</td>
<td>10</td>
</tr>
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</table>

ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, and GCA = giant cell arteritis
Table 2 Giant cell arteritis (GCA) was defined using The American College of Rheumatology (1990) criteria for the classification of giant cell arteritis. The absence of both lymphocytes and multinucleated giant cell infiltrates in the sample led to the categorization of a sample as negative. The histopathological findings in arteries from control patients and arteries from patients with GCA are listed in this table.

<table>
<thead>
<tr>
<th>Presence of multinucleated giant cells</th>
<th>Control n = 10</th>
<th>GCA n = 10</th>
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<tr>
<td>Yes</td>
<td>0</td>
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<tr>
<td>No</td>
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<table>
<thead>
<tr>
<th>Internal elastic lamina degeneration</th>
<th>Control</th>
<th>GCA</th>
</tr>
</thead>
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<tr>
<td>Intact</td>
<td>5</td>
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<tr>
<td>Focal rupture</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Rupture &lt; 50% of the vessel circumference</td>
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<td>8</td>
</tr>
<tr>
<td>Rupture &gt; 50% of the vessel circumference</td>
<td>1</td>
<td>0</td>
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</table>

<table>
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<tr>
<th>Intimal hyperplasia</th>
<th>Control</th>
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<tr>
<td>Absent</td>
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<td>0</td>
</tr>
<tr>
<td>0-25% lumen occlusion</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>25-50% lumen occlusion</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>&gt; 50% lumen occlusion</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Multinucleated giant cells</td>
<td>0</td>
<td>9</td>
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**DAB staining intensity**

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<tr>
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</tr>
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DAB = Diaminobenzidine and GCA = giant cell arteritis.
Figure legends

Figure 1: Representative example of hematoxylin-eosin-stained sections of a temporal artery from (A) a control patient and (B) a patient with giant cell arteritis (GCA). Note the intimal hyperplasia with reduction of the lumen, internal elastic lamina degeneration, band-shaped inflammatory infiltrates of immune cells, and the focal necrosis in the artery with GCA. (Original magnification x 50, x 200 and x 400.)

Figure 2: Immunostaining with antibodies for angiotensin II type 1 (AT1) receptors in temporal arteries from the control patients and the patients with giant cell arteritis (GCA). The top panels show representative examples of immunohistological images and the bottom panels show the AT1 receptor immunostaining intensities, arbitrary units. The results are shown as mean values ± standard deviation (SD). Statistical analyses were performed using Student’s t-test. Significance was defined as P<0.05 (*). Note that the AT1 receptor expression is higher in both the smooth muscle cell layer and in the inflammatory cells in the temporal artery from the patients with GCA. (Original magnification x 50, x 200 and x 400.)

Figure 3:
Representative examples of immunohistological images showing immunostaining for angiotensin II type 2 (AT2) receptors in temporal arteries from the control patients and patients with giant cell arteritis (GCA). The bottom panels show the AT2 receptor immunostaining intensities, arbitrary units. The results are shown as mean values ± standard deviation (SD). Statistical analyses were performed using Student’s t-test in which
significance is defined as p<0.05. N.S. denotes no significant difference. (Original
magnification x 50 and x400.)