Angiotensin II Stimulates Intercellular Adhesion Molecule-1 (ICAM-1) Expression by Human Vascular Endothelial Cells and Increases Soluble ICAM-1 Release In Vivo

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Background—We evaluated whether angiotensin II (Ang II) influenced intercellular adhesion molecule (ICAM)-1 expression by human vascular endothelial cells derived from umbilical cord veins (HUVECs) and plasma soluble ICAM-1 levels in vivo.

Methods and Results—Cultured HUVECs were incubated with Ang II (from $10^{-9}$ to $10^{-6}$ mol/L) with or without candesartan and PD12319 (inhibitors of Ang II AT$_1$ and AT$_2$ receptors, respectively) for various times up to 4 hours. Total RNA was then extracted from HUVECs, and Northern blots were probed with a 1.9-kb ICAM-1 cDNA fragment. HUVEC supernatants were used to assess soluble ICAM-1 release by ELISA. Northern blot analysis detected a strong increase of ICAM-1 mRNA after 2-hour incubation with Ang II. The response was inhibited by candesartan. Soluble ICAM-1 release by HUVECs also increased ($P<0.002$) after 2-hour Ang II stimulation. In vivo, Ang II (at an initial rate of 1.0 ng · kg$^{-1}$ · min$^{-1}$, to be increased each 30 minutes by 2.0 ng · kg$^{-1}$ · min$^{-1}$ to the final rate of 7.0 ng · kg$^{-1}$ · min$^{-1}$) was infused in 8 normotensive and 12 essential hypertensive individuals. In the latter, Ang II was reinfused after 4 weeks on either placebo ($n=5$), losartan (50 mg UID, $n=5$), or atenolol (50 mg UID, $n=4$) treatment. Plasma soluble ICAM-1 levels increased after Ang II infusion in hypertensives and normotensives ($P<0.0001$ after 90 minutes). Losartan reduced baseline soluble ICAM-1 levels ($P<0.05$) and Ang II–related ICAM-1 increments.

Conclusions—Ang II upregulates ICAM-1 expression by HUVECs and stimulates in vitro and in vivo soluble ICAM-1 release. AT$_1$ receptor blockade inhibits such endothelial effects of Ang II. (Circulation. 1999;100:1646-1652.)

Key Words: cell adhesion molecules ■ endothelium ■ cells ■ angiotensin

The endothelial adhesion intercellular adhesion molecule-1 (ICAM-1) modulates leukocyte adhesion to the vascular endothelium, and its upregulation is suggested to play a critical role in atherogenesis.1 Accordingly, ICAM-1 upregulation was demonstrated in endothelial cells covering human atheromas1-3 and correlated with plaque intimal T-lymphocyte density.4 Several data suggest a linkage between renin system overactivity and human atherosclerosis.5 Such linkage seems to be secondary to the vascular effects of angiotensin II (Ang II), ie, stimulation of plasminogen activator inhibitor-1,6 endothelin-1,7 and free radical production,8 as well as endothelial9 and vascular smooth muscle cell proliferation.10 In addition, Ang II augmented the expression of the endothelial adhesion E-selectin by human coronary endothelial cells and stimulated leukocyte adhesion to the same cells.11

In view of a possible relationship between the renin system and ICAM-1, we investigated the effects of Ang II on expression and regulation of ICAM-1 and soluble ICAM-1 secretion in human vascular endothelial cells derived from umbilical cord veins (HUVECs). We also assessed the effects of intravenous Ang II infusion on plasma soluble ICAM-1 concentrations in humans.

Methods

In Vitro Studies

HUVECs were obtained as previously described.12 Culture purity (>99%) was evaluated by fluorescence-activated cell sorting after CD31 labeling. Confluent HUVECs were incubated alone or with Ang II $10^{-8}$, $10^{-7}$, and $10^{-9}$ mol/L (Sigma Chemical Co) in culture medium containing 20% FCS for various periods of time up to 24 hours. Because the time course of protein expression did not increase after 4 hours, in subsequent experiments HUVECs were incubated for various times up to 4 hours with either enalaprilat (Sigma) ($10^{-4}$, $10^{-5}$, and $10^{-6}$ mol/L), the AT$_1$ receptor inhibitor candesartan cilexetil (donated by Takeda SpA, Italy) ($10^{-5}$, $10^{-6}$, and $10^{-7}$ mol/L), or the AT$_2$ receptor inhibitor PD12319 (donated by Parke-Davis SpA, Italy) ($10^{-4}$, $10^{-5}$, and $10^{-6}$ mol/L) added 30 minutes before Ang II. Tumor necrosis factor (TNF)-α (1 000 U/mL)–stimulated HUVECs served as positive and unstimulated HUVECs...
as negative controls. HUVECs were also incubated with increasing concentrations of TNF-α (from 0.1 to 1.000 U/mL).

**RNA Isolation and Northern Analysis**

Northern analysis was performed as previously described. After extraction, 20 μg of total RNA was fractionated in formaldehyde-denaturing agarose gel and transferred to Hybond-N filters (Amer sham Laboratories) by capillary blotting. Hybridization was with 32P-labeled DNA probes in 50% formamide, 5×SSC (1×SSC=0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate), 0.02 mol/L sodium phosphate pH 7.2, 5×Denhardt’s solution, 0.2% sodium lauryl phosphate, 10% dextran sulfate, and 0.1 mg/mL denatured sheared salmon sperm DNA at 42°C for 18 hours. Filters were washed in 2×SSC, 0.25% sodium lauryl sulfate for 30 minutes with 2 changes and then for 60 minutes in 0.2×SSC, 0.25% sodium lauryl sulfate with 3 changes at 45°C. Autoradiograms were developed after 2 days of exposure. Hybridization to study ICAM-1 gene expression was performed with a 1.9-kb cDNA clone (donated by Dr Brian Seed, Department of Molecular Biology, Harvard Medical School, Massachusetts General Hospital, Boston, Mass).

**Cell ELISA**

HUVECs were washed twice in Mg2+-Ca2+-free PBS. Cell pellets were resuspended in 1 mL hypotonic extraction buffer (1.21 g/L Tris-HCl, 0.029 g/L CaCl2, pH 7.2). Cell lysates were then obtained by a freeze-thawing procedure, and nuclei were spun out by centrifugation at 800g. Supernatants were saved for ELISA, and protein content was measured by the Lowry method.

For ELISA, several dilutions of supernatant were incubated overnight (37°C) in flat-bottomed 96-well plates. Dried supernatants were incubated with PBS/0.1% BSA (pH 7.2) at 37°C for 1 hour, then washed twice with PBS/1% BSA, and an excess amount of anti-ICAM-1 antibody (donated by Dr Allan McClelland, Molecular Therapeutics, Inc, Miles Research Center, West Haven, Conn) was added for 1 hour at 37°C, followed by additional washing. Horseradish peroxidase–conjugated goat anti-mouse IgG (Zymed Laboratories) was added for 1 hour at 37°C and then washed out. The substrate used for colorimetric assay was o-phenylenediamine in phosphate-citrate buffer, pH 5.0. Color development was stopped after 15 minutes of incubation by addition of 2 mol/L H2SO4. Absorbance at 492 nm was determined by EAR 400 ELISA reader (SLT-Laboratory Instruments). The data presented are means of triplicate determinations. Intra-assay variability was 5±1%, and interassay 4±2%.

**Immunocytochemistry**

Adherent HUVECs were immunostained as previously described. Briefly, HUVECs were fixed for 5 minutes at room temperature in 1% paraformaldehyde, rinsed in TBS (0.05 mol/L Tris buffer/NaCl, pH 7.6), treated with 0.01% Triton X-100 in TBS, and incubated for 30 minutes with heat-inactivated normal human AB serum diluted 1:10 in TBS. Cells were then incubated for 1 hour with anti-ICAM-1 monoclonal antibody (Dako G108), rabbit-to-mouse IgG1 (Dako), and APAAP complex (Dako) in sequence. Naphthol AS-BI phosphate (Sigma-Aldrich) was used as substrate, and New Fuxin (Merck) was used to develop positivity. Cells treated with the second antibody and the APAAP complex alone and sections treated with irrelevant isotype-matched monoclonal antibodies served as controls.

**Soluble ICAM-1 in HUVEC Supernatants**

HUVEC supernatants (50 μL) were taken before and after each experiment for soluble ICAM-1 determination by ELISA (R&D Systems). To exclude influences of Ang II on ICAM-1 measurement, Ang II (10−3, 10−4, 10−5, and 10−6 mol/L) was added to supernatants from unstimulated HUVECs. Ang II did not influence ICAM-1 measurements by ELISA (differences between added and unadded supernatants were <4%).

**In Vivo Studies**

Informed consents were requested from healthy subjects (5 men and 3 women) and 12 never-treated essential hypertensive outpatients (7 men and 5 women). Entry criteria for hypertensives were age 35 to 55 years, supine systolic/diastolic blood pressure levels between 160/95 and 179/114 mm Hg, normal glucose and lipid metabolisms, no atherosclerotic lesions in explorable arteries, and absence of concomitant diseases, including allergic diathesis.

**Procedures**

Patients and control subjects were given a diet containing 120 mmol sodium and 80 mmol potassium per day. Adherence to the diet was checked by evaluation of 24-hour urinary sodium and potassium excretion. After 1 week on the diet and an overnight fast, at 8 AM, 2 intravenous lines were inserted in forearm veins for withdrawal of blood and infusions, respectively. After the subject had spent 1 hour in the supine position, either Ang II (Clinalfa AG) dissolved in 50 mL isotonic saline or placebo (50 mL isotonic saline) was infused by use of a peristaltic pump (Abbot-Shaw Life Care Pump). Ang II was infused at an initial rate of 1.0 g kg−1 min−1 for 30 minutes, to be augmented by 2.0 g kg−1 min−1 each 30 minutes until the final rate of 7.0 g kg−1 min−1 was reached and infused for 30 minutes.

Blood samples for determination of circulating ICAM-1 levels and leukocyte counts were taken at baseline, each 30 minutes during the infusion, and after a 1-hour recovery period. Plasma renin activity and aldosterone levels were assessed at the same intervals. Blood pressure and heart rate were recorded each 10 minutes.

After a further week on the above diet, subjects who received placebo were infused with Ang II and vice versa, according to a randomized, single-blind, crossover protocol. Then, hypertensives were randomized to receive either oral placebo (n=3), losartan (50 mg UID, n=5), or atenolol (50 mg UID, n=4) for 4 weeks, and Ang II infusions were repeated in all patients according to the above protocol.

**Laboratory Measurements**

Plasma soluble ICAM-1 concentrations were determined by trildecate by ELISA (R&D Systems). Interassay and intra-assay coefficients of variation were 5±2% and 3±2%, respectively. To exclude influences of Ang II on the ICAM-1 assay, Ang II (10−5, 10−4, 10−3 mol/L) was added to randomly selected plasma samples from 5 hypertensives and 5 control subjects. Plasma soluble ICAM-1 ranged from 90 to 181 μg/L in samples with Ang II and from 89 to 185 μg/L in samples with Ang II. Cumulative differences between samples with and without Ang II were <5%. Plasma renin activity and aldosterone levels were assessed by radioimmunoassay (Sorin).

**Statistical Analysis**

Differences among groups were tested for significance by 1-way ANOVA followed by Bonferroni’s test and the Newman-Keuls test for pairwise comparisons. Multiple comparisons were analyzed by ANOVA followed by post hoc analysis to adjust the significance level. Linear regression and correlation were used to evaluate relationships between variables. Descriptive parameters were tested for significance by the χ2 method. Statistical significance was considered as a value of P<0.05. Data are mean±SD.

**Results**

**In Vitro Data**

Northern analysis detected a significant increment of ICAM-1 mRNA expression in HUVECs after 2 hours of incubation with Ang II (Figure 1A). Similar findings were observed for soluble ICAM-1 in HUVEC supernatants (Figure 1B). Neither enalaprilat nor PD12319 influenced Ang II–induced ICAM-1 mRNA. Candesartan cilexetil abolished
After the first Ang II infusion, hypertensives were randomly divided into 3 groups (Table 2) and assigned to receive either placebo (n=3), losartan (50 mg/d, n=5), or atenolol (50 mg/d, n=4) treatments for 4 weeks. Then Ang II infusions were repeated as above.

Atenolol and losartan decreased blood pressure levels (Table 2). Neither placebo (Figure 3A) nor atenolol (Figure 3B) modified plasma ICAM-1 levels. Losartan significantly (P<0.05) reduced baseline soluble ICAM-1 levels and blunted ICAM-1 response to Ang II (Figure 3C). After losartan, changes of leukocyte count during Ang II infusion were not significant.

**Discussion**

The present report shows that Ang II upregulates ICAM-1 gene expression and stimulates soluble ICAM-1 release by HUVECs. Modulation of ICAM-1 expression and secretion by Ang II was blocked by selective AT1, but not AT2 receptor antagonism. Ang II also increased plasma soluble ICAM-1 levels in healthy volunteers and essential hypertensives. Four-week treatment with losartan but not atenolol or placebo reduced baseline and Ang II-stimulated plasma ICAM-1 concentrations. Therefore, our data indicate that Ang II regulates ICAM-1 expression in the living endothelium.

In contrast to our data, a previous study failed to demonstrate significant Ang II-mediated increments of ICAM-1 expression in human microvascular endothelial cells derived from the coronary system.

Discrepancies between previous reports and our data are due to different cell models. Microvascular endothelial cells of the human coronary system are prone to selectively express adhesion molecules of the selectin family rather than the immunoglobulin superfamily. Accordingly, these cells express E-selectin after Ang II stimulation. In addition, they express L-selectin, an adhesion molecule that is not expressed by HUVECs and epicardial coronary endothelial cells. Moreover, membrane ICAM-1 expression increased 2-fold in cultured epicardial coronary endothelial cells after Ang II stimulation (10^{-8} mol/L). Thus, discrepancies between HUVEC and microvascular endothelial cell responses to Ang II simply reflect cell specificity. Concordantly, ICAM-1 mRNA levels increased 7.1-fold in rat tubular cells after 1-hour incubation with Ang II.

The increment of soluble ICAM-1 release by HUVECs after Ang II stimulation also supports the contention that Ang II regulates ICAM-1 expression in vitro. Soluble ICAM-1 was released by cultured HUVECs, human melanoma cells, and keratinocytes after cytokine stimulation. Soluble

**Table 1. General Characteristics and Baseline Plasma Soluble ICAM-1 Levels in Hypertensive and Normotensive Groups**

<table>
<thead>
<tr>
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<th>Hypertensives</th>
<th>Control Subjects</th>
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<tr>
<td>n</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.2±2.8</td>
<td>47.4±2.3</td>
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<tr>
<td>Sex, M/F</td>
<td>7/5</td>
<td>5/3</td>
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<td>Body mass index, kg/m²</td>
<td>24.7±0.9</td>
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<td>Systolic blood pressure, mm Hg</td>
<td>170.7±3.2</td>
<td>116.7±4.3</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>101.8±3.4</td>
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<td>Serum glucose, mmol/L</td>
<td>4.9±0.1</td>
<td>4.7±0.3</td>
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<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>4.7±0.3</td>
<td>4.6±0.2</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.1±0.2</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>ICAM-1, µg/L</td>
<td>147.1±7.5</td>
<td>141.5±6.5</td>
</tr>
<tr>
<td>Urinary albumin excretion, µg/min</td>
<td>15.8±1.6</td>
<td>13.8±1.7</td>
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</tbody>
</table>

Data are mean±SD.

*P<0.0001 vs control.
†P<0.01 vs control.

The stimulatory action of Ang II on ICAM-1 mRNA (Figure 1C) and soluble ICAM-1 secretion (Figure 1D) showed the strongest ICAM-1 expression (Figure 1G).

Ang II–stimulated adherent cells displayed a higher level of ICAM-1 mRNA expression than unstimulated control preparation (Figure 1E and 1F, respectively). TNF-α–stimulated cells showed the strongest ICAM-1 expression (Figure 1G).

**In Vivo Data**

Plasma soluble ICAM-1 levels did not differ between hypertensives and normotensives (Table 1). Ang II infusion did not modify blood pressure levels in hypertensives or normoten-

The present report shows that Ang II upregulates ICAM-1 gene expression and stimulates soluble ICAM-1 release by HUVECs. Modulation of ICAM-1 expression and secretion by Ang II was blocked by selective AT1, but not AT2 receptor antagonism. Ang II also increased plasma soluble ICAM-1 levels in healthy volunteers and essential hypertensives. Four-week treatment with losartan but not atenolol or placebo reduced baseline and Ang II-stimulated plasma ICAM-1 concentrations. Therefore, our data indicate that Ang II regulates ICAM-1 expression in the living endothelium.

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**Figure 1. A, Northern blot analysis of 20 µg of total RNA from human endothelial cells derived from HUVECs in presence and absence of Ang II 10^{-6} mol/L for various times up to 4 hours. Significant RNA message increase was detected after 2 hours of incubation. B, Increase of soluble ICAM-1 in HUVEC supernatants after cell incubation alone (●) or with Ang II 10^{-7} mol/L (●). 10^{-6} mol/L (●), or 10^{-5} mol/L (●), as detected by ELISA (data are mean±SD of 4 different experiments). TNF-α 10^{-6} mol/L served as control. C, Complete blockade of ICAM-1 expression by cultured HUVECs after incubation with Ang II 10^{-6} mol/L and candesartan 10^{-5} mol/L. D, Soluble ICAM-1 secretion by cultured HUVECs incubated alone (●) or with Ang II 10^{-7} mol/L (●), Ang II 10^{-6} mol/L+candesartan 10^{-5} mol/L (●), or candesartan alone (●). Data are given as mean±SD of 4 different experiments. E, F, and G, Immunocytochemistry showed a higher level of ICAM-1 expression in Ang II–stimulated (10^{-6} mol/L) HUVECs (E) than in unstimulated ones (F). G, Positive control with TNF-α 10^{-6} mol/L. Significances in B: a, P<0.003 vs control; b, P<0.002 vs control; c, P<0.0005 vs control; d, P<0.0004 vs control; e, P<0.0007 vs control. TNF-α–stimulated soluble ICAM-1 secretion was higher than Ang II–stimulated secretion (10^{-6} and 10^{-7} mol/L) after both 2 and 4 hours (P<0.05). Significance in D: f, P<0.002 vs control.**
ICAM-1 release by keratinocytes was associated with expression of membrane ICAM-1 and derived from proteolytic cleavage of the latter and not by alternatively spliced ICAM-1 mRNA (as demonstrated by Northern blot analysis and reverse transcriptase–polymerase chain reaction). Moreover, proteolytic inhibitors (iodoacetamide and E-64) blocked soluble ICAM-1 release but not membrane ICAM-1 expression by keratinocytes.

Our in vivo findings demonstrated that plasma soluble ICAM-1 concentrations did not differ between hypertensives and normotensives. Figure 2. Top, Time course of circulating soluble ICAM-1 response to Ang II (at an initial rate of 1.0 ng · kg⁻¹ · min⁻¹ to be increased each 30 minutes by 2.0 ng · kg⁻¹ · min⁻¹ to final rate of 7.0 ng · kg⁻¹ · min⁻¹) (open symbols) or placebo (50 mL isotonic saline) (solid symbols) in 12 essential hypertensives (left) and 8 normotensives (right). Bottom, Changes of leukocyte count during above Ang II infusions in same groups (hypertensives, left; control subjects, right). Symbols as in top panel. All data are mean ± SD. a, P < 0.0001 vs time 0; b, P < 0.002 vs time 0; c, P < 0.0001 vs time 0; d, P < 0.03 vs time 0.

**TABLE 2.** General Characteristics and Circulating Soluble ICAM-1 Concentrations of 3 Hypertensive Subgroups Before Randomization to Atenolol (50 mg/d), Losartan (50 mg/d), or Placebo Treatments Over a Period of 4 Weeks

<table>
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<th>Placebo</th>
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<th>Atenolol</th>
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<td>n</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.7 ± 1.1</td>
<td>46.8 ± 2.8</td>
<td>45 ± 3.8</td>
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<tr>
<td>Sex, M/F</td>
<td>2/1</td>
<td>3/2</td>
<td>2/2</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>25.3 ± 0.6</td>
<td>24.8 ± 0.83</td>
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<td>Systolic blood pressure, mm Hg</td>
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<tr>
<td>Baseline</td>
<td>171.3 ± 3.0</td>
<td>171.0 ± 4.0*</td>
<td>170.1 ± 2.82*</td>
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<tr>
<td>Posttherapy</td>
<td>165.3 ± 3.0</td>
<td>154.8 ± 4.8</td>
<td>148.5 ± 1.9</td>
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<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>100.0 ± 2</td>
<td>102.8 ± 4.1*</td>
<td>102.1 ± 3.7*</td>
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<tr>
<td>Posttherapy</td>
<td>96.3 ± 1.1</td>
<td>88.0 ± 4.5</td>
<td>88.5 ± 5.5</td>
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<td>Serum glucose, mmol/L</td>
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<td>Serum total cholesterol, mmol/L</td>
<td>4.5 ± 0.5</td>
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<td>Serum triglycerides, mmol/L</td>
<td>1.0 ± 0.2</td>
<td>1.2 ± 0.14</td>
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<td>ICAM-1, µg/L</td>
<td>148.7 ± 3.05</td>
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<td>Urinary albumin excretion, µg/min</td>
<td>15.3 ± 1.1</td>
<td>16 ± 2</td>
<td>16 ± 1.7</td>
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Blood pressure changes after each treatment are also given. Data are mean ± SD. *P < 0.0001 vs after therapy.
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Figure 3. Effects of Ang II infusion (at an initial rate of 1.0 ng·kg⁻¹·min⁻¹) on circulating soluble ICAM-1 levels in essential hypertensives before (fold) and after (fold) 4 weeks on placebo (n=3, A), losartan 50 mg UID (n=5, B), or atenolol 50 mg UID (n=4, C) treatments (mean±SD). A, P<0.003 vs time 0; b, P<0.0002 vs time 0; c, P<0.0004 vs time 0; d, P<0.0001 vs time 0; e, P<0.005 vs baseline; f, P<0.002 vs time 0; g, P<0.003 vs baseline; h, P<0.0001 vs baseline.

and normotensives and increased with Ang II infusion in both groups. In hypertensives, plasma soluble ICAM-1 levels changed after losartan but not placebo or atenolol treatments.

The normality of circulating ICAM-1 levels in hypertensives is in agreement with previous data. By contrast, a recent report described only slight increments (11%) of plasma soluble ICAM-1 levels in 8 young, healthy volunteers receiving graded Ang II infusions. The reasons for this discrepancy are unclear. Possible influences of atherosclerotic lesions, impaired glucose tolerance, allergies, and other conditions known to affect soluble ICAM-1 release in vivo were excluded in our study. Because shear-stress increments augment soluble ICAM-1 release in vitro, we used nonpressor Ang II doses. Because vascular responses to Ang II are affected by changes in sodium intake, this latter was maintained at a constant level in all subjects. Thus, the reported lack of soluble ICAM-1 response to Ang II infusion might reflect excess salt intake and the consequent downregulation of Ang II receptors.

Ang II-induced ICAM-1 expression occurred within 1 hour of incubation. Furthermore, in vivo data clearly demonstrated that plasma ICAM-1 concentrations increase within 1 hour after exercise and oral glucose loading. Therefore, the time course of circulating soluble ICAM-1 responses to Ang II infusion indicates that in vitro data cannot simply be transposed to in vivo settings. With regard to the rapid return to baseline of plasma soluble ICAM-1 level after the end of Ang II infusion, excess soluble ICAM-1 is adsorbed by target cells in vivo and rapidly cleared from the circulation. Accordingly, a rapid return to baseline plasma soluble ICAM-1 levels has also been observed after glucose ingestion.

Our study also confirms that Ang II increases leukocyte count. In this regard, Ang II induces the production of a leukocyte chemoattractant from bovine aortic and human endothelial cells. Furthermore, AT₁ receptors are expressed on the leukocyte surface, where they might modulate proliferative and migratory responses to cytokines. Thus, either activation of an endothelium-derived chemoattractant or stimulation of an AT₁ receptor–dependent pathway activating a proliferative response, or both, might be responsible for the increased leukocyte count due to Ang II. Concordantly, Ang II–related changes in leukocyte count were not significant after losartan. Although this hypothesis looks extremely intriguing, baseline leukocyte count was not affected by losartan, and AT₁ receptor inhibitors are not known to induce significant changes in leukocyte count. Therefore, the pathogenesis of Ang II–related changes in leukocyte count remains elusive.

In conclusion, we showed that Ang II upregulated ICAM-1 expression and stimulated soluble ICAM-1 secretion by cultured HUVECs via AT₁ receptors. Ang II also promoted soluble ICAM-1 release in vivo before but not after AT₁ receptor blockade. The role of ICAM-1 in experimental atherosclerosis is well recognized. Growing evidence suggests that ICAM-1 upregulation is a fundamental step in human atherogenesis. Thus, our data establish a new link between Ang II and vascular damage in humans.

Acknowledgments

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References


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