Brief Rapid Communication

Vasoconstriction to Endogenous Endothelin-1 Is Increased in the Peripheral Circulation of Patients With Essential Hypertension

Stefano Taddei, MD; Agostino Virdis, MD; Lorenzo Ghiadoni, MD; Isabella Sudano, MD; Massimo Notari, MD; Antonio Salvetti, MD

Background—In humans, endothelin (ET)-1 could be implicated in the pathophysiology of several cardiovascular diseases, including essential hypertension. We therefore evaluated the role of ET-1 in control of vascular tone in essential hypertension.

Methods and Results—We used strain-gauge venous plethysmography to test changes in forearm blood flow induced by intrabrachial infusion of TAK-044 (10, 30, and 100 μg · 100 mL⁻¹ · min⁻¹), an ETₐ/ETₐ receptor antagonist, or sodium nitroprusside (1 and 2 μg · 100 mL⁻¹ · min⁻¹), a vasodilator that acts on smooth muscle cells, in hypertensive patients and healthy controls (n = 10 in each group). The NO pathway was also evaluated by infusion of N⁶-monomethyl-L-arginine, (L-NMMA; 10, 30, and 100 μg · 100 mL⁻¹ · min⁻¹), an NO synthase inhibitor, and norepinephrine (3, 9, and 30 ng · 100 mL⁻¹ · min⁻¹) as control. Immunoreactive plasma ET-1 was measured by radioimmunoassay. In hypertensive patients, TAK-044 caused a vasodilation that was significantly (P < 0.01) increased compared with normotensive subjects. Moreover, vasoconstriction to L-NMMA was significantly (P < 0.01) decreased in hypertensive patients compared with controls. In contrast, the vascular responses to sodium nitroprusside and norepinephrine, as well as levels of immunoreactive plasma ET-1, were similar in hypertensive patients and controls. In the study population, vasodilation to TAK-044 and vasoconstriction to L-NMMA showed an inverse correlation (r = −0.56, P < 0.05).

Conclusions—These results indicate that TAK-044 caused a greater degree of vasodilation in the forearm vessels of essential hypertensive patients compared with normotensive subjects, an alteration associated with decreased tonic NO release. (Circulation. 1999;100:1680-1683.)

Key Words: vasodilation ■ hypertension ■ nitric oxide ■ endothelin

Endothelin-1 (ET-1), a 21-amino-acid isopeptide generated by the vascular endothelium and characterized by sustained and potent vasoconstrictor action, acts through specific receptors termed ETₐ and ETₐ. ETₐ receptors are represented only on smooth muscle cells to evoke contractions and promote growth. In contrast, ETₐ receptors are located both on smooth muscle cells to evoke contractions and on endothelial cells to induce relaxation by production of nitric oxide (NO).

In humans, ET-1 could be implicated in the pathophysiology of several cardiovascular diseases, including essential hypertension. Bosentan, a mixed ETₐ/ETₐ receptor antagonist, significantly lowered blood pressure values in patients with essential hypertension, which suggests a role for this peptide in the pathogenesis of hypertension. However, a clear demonstration of a disturbance in ET-1–mediated vascular control is still lacking in essential hypertension.

In the present study, we compared forearm vasodilation to TAK-044, a combined ETₐ/ETₐ receptor antagonist, in healthy control subjects and patients with essential hypertension to indirectly assess the contribution of endogenous ET-1 to vascular tone in both conditions. Moreover, the activity of the L-arginine–NO pathway was also evaluated.

Methods

The study population included 10 normotensive control subjects (age 51.2 ± 6.4 years; blood pressure 121.4 ± 3.7/82.2 ± 3.2 mm Hg) and 10 matched never-treated patients with essential hypertension (age 50.6 ± 6.8 years; blood pressure 160.2 ± 6.2/101.6 ± 2.8 mm Hg) characterized by no history of smoking or drinking and by similar values of plasma glucose, total and LDL cholesterol, and creatinine clearance (all within normal ranges). The protocol was approved by the ethics committee of the University of Pisa, and all patients gave written consent to participate in the study.

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Briefly, the brachial artery was cannulated for drug infusion at systematically ineffective rates and for monitoring of intra-arterial blood pressure and heart rate. Forearm blood flow (FFB) was measured in both forearms by strain-gauge venous plethysmography. Circulation to the hand was excluded 1 minute before FBF measurement by inflation of a pediatric cuff around the wrist at suprasystolic pressure. Forearm volume was measured according to the water-displacement method. Details concerning this method have been published previously.

To assess ET-1 contribution to vascular tone, TAK-044 (Takeda Chemical Industries Ltd., a specific inhibitor of ET$_A$/ET$_B$ receptors,$^{7,8,10}$ was infused into the brachial artery of normotensive and essential hypertensive subjects and patients with essential hypertension (10, 30, and 100 $\mu$g per 100 mL of forearm tissue/min; 10 minutes for each dose). The IC$_{50}$ for TAK-044 is 3.8 and 130 mmol/L for ET$_A$ and ET$_B$ receptors, respectively, in vitro and is similar in vivo.$^9$ Moreover, the integrity of the NO pathway was evaluated by administration of intrabrachial N$^\text{N}$-monomethyl-L-arginine (L-NMMA; Clinalfa AG), a specific antagonist for NO synthases$^{12}$ (10, 30, and 100 $\mu$g per 100 mL of forearm tissue/min; 5 minutes for each dose). As a control for nonspecific vascular responses, vasodilation to sodium nitroprusside (SNP; Malesci), a direct smooth muscle cell relaxant compound (1 and 2 $\mu$g per 100 mL forearm tissue$^{-1}$·min$^{-1}$; 5 minutes for each dose), was also evaluated. The infusion sequence was randomized, and a 60-minute washout period was allowed between each dose-response curve. However, TAK-044 was always administered as the last infusion. In each subject, a venous blood sample was obtained for assay of plasma immunoreactive ET concentrations (by radioimmunoassay).$^{13}$

Data were analyzed in terms of forearm vascular resistances (FVRs) (calculated as the ratio between intraarterial mean pressure and FBF and expressed as standard units). Clinical characteristics of study subjects were compared by the paired Student $t$ test. Dose-response curves were analyzed by ANOVA for repeated measures, and Scheffé’s test was applied for multiple comparison testing. Results are expressed as mean±SD.

## Results

The Table describes mean blood pressure, heart rate, and experimental and contralateral FVR behavior during each infusion.

In healthy subjects, TAK-044 caused a modest increase in FBF (Figure 1) and a decrease in FVR ($-12.3\pm17.2\%$), whereas in patients with essential hypertension, the FBF increase (Figure 1) and FVR decrease ($-47.2\pm12.1\%$) were significantly ($P<0.01$) greater than for normotensive controls. L-NMMA caused a decrease in FBF (Figure 1), which was significantly ($P<0.01$) reduced in essential hypertensive patients (FVR increase $41.4\pm14\%$) compared with normotensive subjects (FVR increase $95.3\pm41\%$). Vasodilation to SNP and vasoconstriction to NE were similar in normotensive subjects and hypertensive patients. Contralateral FVR did not significantly change throughout the study (Table).

With regard to the entire study population, vasodilation to TAK-044 was negatively ($r=-0.56, P<0.05$) correlated with vasoconstriction to L-NMMA (Figure 2). Plasma immunoreactive ET was similar in normotensive subjects ($4.9\pm0.1$ pg/mL) and hypertensive patients ($4.3\pm0.1$ pg/mL).

## Discussion

The present results show that TAK-044, a combined ET$_A$/ET$_B$ receptor antagonist, caused a greater degree of vasodilation in the forearm vasculature of patients with essential hypertension than in normotensive control subjects, whereas the response to SNP, a direct relaxant compound, was similar in

### Table 1: Hemodynamic Parameters During Infusion of TAK-044, SNP, L-NMMA, and NE

<table>
<thead>
<tr>
<th></th>
<th>Normotensive Subjects</th>
<th>Essential Hypertensive Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBP, mm Hg</td>
<td>HR, bpm</td>
</tr>
<tr>
<td>TAK-044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>90.5±4.2</td>
<td>73.0±3.9</td>
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<tr>
<td>10 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.5±4.3</td>
<td>72.8±4.4</td>
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<tr>
<td>30 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.0±4.3</td>
<td>72.6±4.2</td>
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<tr>
<td>100 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.0±4.6</td>
<td>72.5±4.0</td>
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<tr>
<td>SNP</td>
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</tr>
<tr>
<td>Baseline</td>
<td>90.4±4.2</td>
<td>72.4±4.6</td>
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<tr>
<td>1 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.5±4.3</td>
<td>72.2±4.6</td>
</tr>
<tr>
<td>2 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.5±4.3</td>
<td>72.6±4.4</td>
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<tr>
<td>L-NMMA</td>
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<tr>
<td>Baseline</td>
<td>89.8±4.5</td>
<td>72.9±3.5</td>
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<tr>
<td>10 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>88.8±4.5</td>
<td>72.7±3.8</td>
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<tr>
<td>30 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
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<tr>
<td>100 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.0±4.5</td>
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<tr>
<td>NE</td>
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<tr>
<td>Baseline</td>
<td>89.9±4.7</td>
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<tr>
<td>3 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>89.8±4.5</td>
<td>72.5±4.1</td>
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<tr>
<td>9 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>89.9±4.7</td>
<td>72.9±4.0</td>
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<tr>
<td>30 $\mu$g·100 mL$^{-1}$·min$^{-1}$</td>
<td>90.0±4.8</td>
<td>72.7±4.2</td>
</tr>
</tbody>
</table>

Ex indicates experimental; c, contralateral. *$P<0.01$ or †$P<0.001$ vs baseline.
An alternative explanation may be related to impaired ET-1–mediated NO production in essential hypertensives. As previously demonstrated, intrabrachial TAK-044 only slightly increased FBF in normotensive subjects (≈20%), indicating that in healthy conditions, endothelial ET

mediated NO-dependent vasodilation almost completely counterbalances smooth muscle ET

mediated vasoconstriction. In essential hypertension, ET

receptor stimulation leads to modest NO activation because of the presence of impaired NO availability, thereby unmasking the vasoconstrictor effect of the peptide. In line with this possibility and in agreement with previous evidence, our hypertensive study population was characterized by an impairment in the NO system, because the vasoconstrictor effect of L-NMMA but not that of the unrelated vasoconstrictor NE was decreased compared with healthy controls. Moreover, the existence of a negative and significant correlation between the vasodilating and vasoconstricting responses to TAK-044 and L-NMMA, respectively, seems to indicate an association between increased vasoconstriction to endogenous ET-1 and diminished NO production. Finally, recent evidence indicates that intrabrachial infusion of BQ-788, a selective ET

receptor antagonist, causes vasodilation and vasoconstriction in essential hypertensives and normotensive controls, respectively, further supporting the existence of a differential vascular activity for ET

receptors in healthy conditions and in patients with essential hypertension.

In conclusion, in essential hypertension, the vasoconstrictor activity of endogenous ET-1 is increased compared with healthy conditions, which suggests a possible role for ET-1 in the pathogenesis of hypertension and/or its complications.

References


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