Cycloxygenase Inhibition Restores Nitric Oxide Activity in Essential Hypertension

Stefano Taddde, Agostino Virdis, Lorenzo Ghiadoni, Armando Magagna, Antonio Salvetti

Abstract To evaluate whether cyclooxygenase constrictor substances can impair nitric oxide–mediated vasodilation in essential hypertension, in seven normotensive subjects (43 ± 4.1 years, BP, 117 ± 6/81 ± 2 mm Hg) and seven essential hypertensive patients (47 ± 5.2 years, BP, 121 ± 8/98 ± 4 mm Hg), we studied forearm blood flow (strain-gauge plethysmography) modifications induced by intrabrachial acetylcholine (0.15, 0.45, 1.5, 4.5, 15 μg 100 mL⁻¹ min⁻¹) in basal conditions, during infusion of N⁶-monomethyl-L-arginine (L-NMMA, 100 μg 100 mL⁻¹ min⁻¹), a nitric oxide synthase inhibitor, or indomethacin (50 μg 100 mL⁻¹ min⁻¹), a cyclooxygenase inhibitor, or simultaneous indomethacin and L-NMMA. In normotensives, vasodilation to acetylcholine was increased by L-arginine (maximum flow increase 539 ± 48%, and 806 ± 61%, respectively) and this effect was unchanged by indomethacin. In hypertensive patients, vasodilation to acetylcholine (maximum flow increase 399 ± 52%) was unchanged by L-arginine but was significantly increased by indomethacin (maximum flow increase 392 ± 56%, P<0.01 versus indomethacin alone). Therefore, cyclooxygenase inhibition restores nitric oxide–mediated vasodilation in essential hypertension, suggesting that cyclooxygenase-dependent substances can impair nitric oxide production.

Key Words • hypertension • endothelium • nitric oxide • endothelium-derived factors • indomethacin

Endothelium plays a major role in the modulation of vascular tone through the production and release of different relaxing and constricting factors acting on the underlying smooth muscle cells. The major endothelium-derived relaxing factor is NO, a labile substance derived from L-arginine by the activity of the enzyme NO synthase. Importantly, this process can be competitively inhibited by L-arginine analogues such as L-NMMA. Moreover, EDCF can negatively interact with the L-arginine pathway, occurring NO destruction. Thus the present study was designed to evaluate whether cyclooxygenase-dependent EDCF can impaire L-arginine–NO pathway and production of cyclooxygenase-dependent EDCF.

However, the possibility exists that these alterations are not abnormalities which are parallely associated with hypertensive disease, but, as demonstrated in certain animal models, EDCF can negatively interact with the L-arginine pathway, causing NO destruction. Thus the present study was designed to evaluate whether cyclooxygenase-dependent EDCF can impair the L-arginine–NO pathway in essential hypertensive patients. Specifically, the investigation focused on assessing whether cyclooxygenase blockade can restore the facilitating or inhibiting effect of L-arginine and L-NMMA, respectively, on the vasodilating response to acetylcholine in essential hypertensive patients.

Methods

Patients

The study population included 14 normotensive control subjects and 14 matched essential hypertensive patients. Subjects with hypercholesterolemia (total cholesterol greater than 5.2 mmol/L), diabetes mellitus, cardiac and/or cerebral ischemic vascular disease, impaired renal function and other major pathologies were excluded from the study. Moreover, subjects or patients smoking more than five cigarettes per day and/or consuming more than 60 g of ethanol (corresponding to half a liter of wine) per day were excluded from the study. In accordance with institutional guidelines, all patients were aware of the investigational

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nature of the study and gave written consent to it. Any pharmacological treatment was discontinued for at least 2 weeks before performing the study.

Subjects, defined as normal according to the absence of familial history of essential hypertension and BP below 140/90 mm Hg, were characterized by mean age of 47.6 ± 4.3 years and BP values of 115.6 ± 6.1/90.3 ± 2.1 mm Hg. Essential hypertensive patients were recruited from among the newly diagnosed cases in our outpatient clinic if they reported the presence of positive family history of essential hypertension, whenever supine arterial BP (after 10 minutes of rest) measured by mercury sphygmomanometer three times at 1-week intervals was consistently found greater than 140/90 mm Hg. Secondary forms of hypertension were excluded by routine diagnostic procedures. Mean age was 50.6 ± 6.6 years and BP values were 152.5 ± 8.7/99.2 ± 3.6 mm Hg. Since the patients were newly diagnosed cases, they were never treated and the known history of hypertension had lasted 2 ± 0.4 years. The demographic and clinical characteristics of the two groups are shown in the Table.

### Experimental Procedure
All studies were performed at 0800 AM after overnight fast with the subjects lying supine in a quiet, air-conditioned room (22°C to 24°C). A polyethylene cannula (21 gauge, Abbott) was inserted into the brachial artery under local anesthesia (2% lidocaine) and connected through stopcocks to a pressure transducer (Model MS20, Electromedics) for systemic mean BP (one third pulse pressure + diastolic pressure) and heart rate monitoring (Model VSM1, Physiocontrol) and for intra-arterial infusions (Model VSM1, Physiocontrol) and for intra-arterial infusions (Model VSM1, Physiocontrol) and for intra-arterial infusions (Model VSM1, Physiocontrol) and for intra-arterial infusions (Model VSM1, Physiocontrol) and for intra-arterial infusions (Model VSM1, Physiocontrol). Drugs infused intrabrachially at 200 μg/100 mL forearm tissue per minute (30 minutes). Washout was allowed between each dose-response curve to intra-arterial acetylcholine.

**Characteristics of Study Subjects (mean±SD)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects (n=14)</th>
<th>Essential Hypertensive Patients (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47.6±4.3</td>
<td>50.6±6.6</td>
</tr>
<tr>
<td>Age range, y</td>
<td>39-56</td>
<td>37-81</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>9/5</td>
<td>10/4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.4±4.1</td>
<td>71.6±5.2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>115.6±6.1</td>
<td>152.5±8.7</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>80.3±2.1</td>
<td>99.2±3.5</td>
</tr>
<tr>
<td>Cardiac mass, g/m²</td>
<td>110.6±7.1</td>
<td>116.4±8.4</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>82.6±6.1</td>
<td>89.4±5.9</td>
</tr>
<tr>
<td>Plasma total cholesterol, mg/dL</td>
<td>189.2±123</td>
<td>193.4±12.8</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mg/dL</td>
<td>42.4±6.8</td>
<td>40.2±6.4</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mg/dL</td>
<td>118.6±10.2</td>
<td>121.4±11.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.8±0.5</td>
<td>22.1±0.6</td>
</tr>
<tr>
<td>FBF, mL/100 mL⁻¹ min⁻¹</td>
<td>3.4±0.4</td>
<td>3.4±0.5</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein, LDL, low-density lipoprotein
L-NMMA (Chinalfia AG), and sodium nitroprusside (Malesci) were obtained from commercially available sources and diluted freshly to the desired concentration by adding normal saline. Sodium nitroprusside was dissolved in glucose solution and protected from light by aluminum foil.

Results

Response to Intrabrachial Acetylcholine and Sodium Nitroprusside

In the overall population, vasoconstriction to acetylcholine was significantly ($P<0.01$) blunted in essential hypertensive patients (FBF rose from 3.4±0.5 to a maximum of 17±3.2 mL/100 mL forearm tissue per minute with the highest dose) compared with normotensive control subjects (FBF rose from 3.4±0.4 to a maximum of 23.9±5.4 mL/100 mL forearm tissue per minute with the highest dose) (Fig 1). In contrast, the vasoconstrictive effect of the endothelium-independent vasodilator sodium nitroprusside was similar in normotensive subjects and essential hypertensive patients (FBF rose from 3.6±0.4 to a maximum of 24±2.9 mL/100 mL forearm tissue per minute with the highest dose and from 3.5±0.4 to a maximum of 23±3.1 mL/100 mL forearm tissue per minute, respectively, NS) (Fig 1).

Effect of Cyclooxygenase Inhibition on Response to Acetylcholine in the Presence of L-NMMA

In this group of normotensive control subjects, L-NMMA infusion caused a decrement in basal FBF (from 3.4±0.4 to 1.9±0.2 mL/100 mL forearm tissue per minute; $P<0.01$) and significantly blunted the vasodilating effect of acetylcholine (saline: from 3.4±0.4 to 26.2±5.6 mL/100 mL forearm tissue per minute, L-NMMA from 1.9±0.2 to 8.3±2.1 mL/100 mL forearm tissue per minute, $P<0.01$ versus acetylcholine alone) (Fig 2). Again, indomethacin did not change either basal FBF (from 3.4±0.4 to 3.4±0.4 mL/100 mL forearm tissue per minute), the response to acetylcholine (from 3.4±0.4 to 26.8±5.4 mL/100 mL forearm tissue per minute), or the inhibiting effect of L-NMMA on vasoconstriction to acetylcholine (from 1.9±0.2 to 8.3±2.5 mL/100 mL forearm tissue per minute) (Fig 2).

In the essential hypertensive patients, L-NMMA infusion caused a decrement in basal FBF (from 3.2±0.6 to 2.3±0.5 mL/100 mL forearm tissue per minute, $P<0.01$) which was significantly smaller than that observed in normotensive control subjects (percent FBF decrease 44% versus 28%, respectively, $P<0.01$). However, the response to acetylcholine (from 3.2±0.5 to 17.7±4.2 mL/100 mL forearm tissue per minute) was not changed by L-NMMA (from 2.3±0.5 to 12.5±3.2 mL/100 mL forearm tissue per minute, NS versus saline) (Fig 2). Indomethacin infusion did not change basal FBF (from 3.1±0.4 to 3.1±0.6 mL/100 mL forearm tissue per minute) Nevertheless, the cyclooxygenase inhibitor increased the response to acetylcholine (from 3.1±0.4 to 22.0±3.3 mL/100 mL forearm tissue per minute, $P<0.01$ versus acetylcholine during saline) (Fig 2). Finally, when the effect of L-NMMA was tested in the presence of indomethacin, the NO synthase inhibitor blunted the vasoconstrictive response to acetylcholine (from 2.3±0.5 to 12.5±3.7 mL/100 mL forearm tissue per minute, $P<0.01$ versus acetylcholine during indomethacin alone) (Fig 2).
Different results were obtained in essential hypertensive patients. Again acetylcholine infusion caused a dose-dependent vasodilation (from 3.6 ± 0.5 to 16.4 ± 2.9 mL/100 mL forearm tissue per minute) which was statistically lower (P < 0.01) than that observed in normotensive control subjects. L-Arginine administration changed neither basal FBF (from 3.6 ± 0.5 to 3.7 ± 0.4 mL/100 mL forearm tissue per minute) or the vasodilating effect of acetylcholine (from 3.7 ± 0.4 to 16.5 ± 3.1 mL/100 mL forearm tissue per minute, NS versus saline) (Fig. 3). Indomethacin did not change basal FBF (from 3.3 ± 0.5 to 3.4 ± 0.4 mL/100 mL forearm tissue per minute), but significantly increased the response to acetylcholine (from 3.4 ± 0.4 to 23.6 ± 3.4 mL/100 mL forearm tissue per minute, P < 0.01 versus saline) (Fig. 2). It is worth noting, finally, that when L-arginine was coinfused with indomethacin the vasodilating effect of acetylcholine was further increased (from 3.4 ± 0.5 to 31.1 ± 4.1 mL/100 mL forearm tissue per minute, P < 0.01 versus acetylcholine in the presence of indomethacin) (Fig. 3).

In both normotensive subjects and essential hypertensive patients, contralateral FBF did not significantly change during the whole study (data not shown).

**Discussion**

Essential hypertension is characterized by endothelial dysfunction. This alteration is further confirmed in the present study since the response to acetylcholine, an endothelium-dependent vasodilator, but not to sodium nitroprusside, a direct smooth muscle cell relaxant, was blunted in essential hypertensive patients compared with matched normotensive control subjects. The mechanisms responsible for the impaired endothelium-dependent vasodilation include an alteration in the L-arginine-NO pathway and production of cycooxygenase-dependent EDCF. That these mechanisms can operate in essential hypertensive patients is confirmed by the present results. Thus, in agreement with previous observations, administration of L-arginine, the substrate for NO synthase, can increase the vasodilating effect of acetylcholine in normotensive subjects while the amino acid is ineffective in essential hypertensive patients. Moreover, L-NMMA, an antagonist of NO synthase, can blunt the response to acetylcholine in control subjects, but not in hypertensive patients. Taken together these results clearly confirm the presence of a defect in the endothelium-derived NO system in essential hypertension, since neither activation nor inhibition of the NO pathway can lead to modifications of the vascular response to the endothelium-dependent vasodilator. It is important to observe that the lack of effect of these compounds on acetylcholine-induced vasodilation is not linked to insufficient infusion rates of either L-arginine or L-NMMA, as already demonstrated by previous evidence obtained in similar experimental conditions.

Moreover, this abnormality does not totally account for the impaired vasodilation to acetylcholine observed in essential hypertension. Thus in hypertensive patients, but not in normotensive subjects, indomethacin increased the response to the endothelium-dependent vasodilator, confirming that, in agreement with previous evidence, the production of cyclooxygenase derivatives can curtail endothelial responses in essential hypertension. It is worth noting that for the first time the alteration in the endothelium-derived NO system and production of cyclooxygenase-dependent EDCF has been demonstrated in the same

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**Effect of Cyclooxygenase Inhibition on Response to Acetylcholine in the Presence of L-Arginine**

In this group of normotensive control subjects, acetylcholine-dependent vasodilation (from 3.5 ± 0.5 to 22.3 ± 3.3 mL/100 mL forearm tissue per minute) was significantly (P < 0.01) increased by the simultaneous infusion of L-arginine (from 3.7 ± 0.3 to 33.5 ± 5.8 mL/100 mL forearm tissue per minute) (Fig. 3). Indomethacin administration failed to affect either the response to acetylcholine infused alone (from 3.6 ± 0.5 to 22.5 ± 3.5 mL/100 mL forearm tissue per minute, P = NS versus acetylcholine during saline) or the facilitating effect of L-arginine on acetylcholine-induced vasodilation (FBF from 3.5 ± 0.5 to 31.9 ± 5.2 mL/100 mL forearm tissue per minute, P = NS versus acetylcholine during saline + indomethacin) (Fig. 2). Of note is that both L-arginine and indomethacin, when infused alone, did not change basal FBF (data not shown).

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**Fig 3** Acetylcholine-induced increase in FBF in the absence and presence of L-arginine (200 µg/100 mL forearm tissue per minute) under control conditions (left) and in the presence of indomethacin (60 µg/100 mL forearm tissue per minute) (right) in normotensive subjects (n=7) (top) and essential hypertensive patients (n=7) (bottom). Data are shown as mean±SEM and expressed as absolute values. Asterisks denote a significant difference between infusion with and without L-arginine (P < 0.05).

In both normotensive subjects and essential hypertensive patients contralateral FBF did not significantly change during the whole study (data not shown).
patients, supporting the possibility that these endothelial alterations coexist in essential hypertension.

However, the main finding of the present study is the demonstration that in essential hypertensive patients cyclooxygenase-dependent EDCF production is restored by indomethacin administration, whereas the simultaneous infusion of L-NMMA with indomethacin abolishes the production of EDCF (endoperoxide or superoxide anions), they can cause the activation of two parallel pathways involving both NO synthase and cyclooxygenase. Cytochrome oxidase activity could lead to the production of NO-inactivating substances (endoperoxides or superoxide anions), thus explaining the absence of effects of L-arginine and L-NMMA on vasodilation to acetylcholine. When cyclooxygenase is blocked by indomethacin and NO breakdown no longer occurs or is at least decreased, it is therefore possible to demonstrate the activity of L-arginine and L-NMMA as observed in normotensive control subjects.

The relationship between cyclooxygenase-dependent EDCF and primary hypertension is of interest. It must be noted that these substances, while causing endothelial dysfunction, do not seem to contribute to an increase in BP values. Thus, in the spontaneously hypertensive rat, treatment by ifetrolan, a thromboxane A2/prostaglandin endoperoxide-receptor blocker, normalized endothelium-dependent relaxations to acetylcholine when cyclooxygenase is blocked by indomethacin and NO breakdown no longer occurs or is at least decreased, it is therefore possible to demonstrate the activity of L-arginine and L-NMMA as observed in normotensive control subjects.

As regards the important issue of the relationship between the duration of essential hypertension and EDCF production, no data are available to understand whether the degree of synthesis of these substances is also dependent on the length of the hypertensive process. In the present study, unfortunately the recruited hypertensive population shows a quite short duration of hypertension and no conclusion can be drawn.

Finally, it is worth noting that these endothelial mechanisms operate mainly when endothelial cells are stimulated by acetylcholine. Thus, in agreement with previous observations, neither L-arginine nor indomethacin (nor the combination of both compounds) can influence basal blood flow in either normotensive subjects or essential hypertensive patients. In contrast, L-NMMA infusion can decrease basal FBF, confirming that NO is basally released in human vasculature and this mechanism is defective in essential hypertension since, as previously demonstrated, L-NMMA-induced vasodilation is blunted in hypertensive patients compared with control subjects. However, the simultaneous infusion of indomethacin with L-NMMA does not change the vasoconstrictor effect of the NO synthase inhibitor suggesting that cyclooxygenase activity does not participate in NO-mediated local regulation of basal flow.

In conclusion, the present results indicate that endothelial dysfunction which is characteristic of essential hypertension is determined by the simultaneous presence of an alteration in the L-arginine–NO pathway and production of cyclooxygenase derivatives. These alterations do not seem to be independent since in essential hypertensive patients, but not in normotensive control subjects, the dysfunctioning NO system seems to be restored or at least improved by cyclooxygenase blockade. Which cyclooxygenase-dependent substances could be responsible for inhibition of the L-arginine NO pathway is, at the present time, under investigation.

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