Abstract—Impaired flow-dependent, endothelium-mediated vasodilation is an early finding in patients with coronary artery disease (CAD). Experimental and some clinical studies observed that angiotensin type-1 receptor antagonists (AT1A) enhance endothelium-dependent relaxation in CAD. The present study was designed to determine whether AT1A improves flow-dependent dilation (FDD) in patients with CAD and, if so, whether bradykinin and NO are involved. High-resolution ultrasound was used to measure radial artery diameter at rest and during reactive hyperemia, causing endothelium-mediated vasodilation. Twenty patients with CAD were randomly assigned to receive intrabrachial infusion of candesartan (800 μg/min) with and without icatibant, a bradykinin B2-receptor antagonist (90 μg/min; group A) or N-monomethyl-L-arginine (L-NMMA), an NO-synthase inhibitor (7 μmol/min; group B). The AT1A candesartan improved FDD by >40%, an effect that was inhibited by icatibant (group A: control, 7.3±0.9; candesartan, 10.3±1.1; candesartan+icatibant, 5.0±0.5%). Similarly, L-NMMA blunted the beneficial effect of candesartan (group B: control, 6.3±0.6; candesartan, 8.9±0.6; candesartan+L-NMMA: 4.7±0.5%; each P<0.01). The angiotensin type-1 receptor antagonist candesartan improves flow-dependent, endothelium-mediated vasodilation in patients with CAD. This effect is inhibited by either icatibant and/or L-NMMA, suggesting that both bradykinin and NO contribute to the vascular effects of AT1-receptor antagonists in this patient population. (Hypertension. 2003;41:1092-1095.)

Key Words: endothelium ■ angiotensin II ■ receptors, angiotensin II ■ angiotensin antagonist ■ bradykinin ■ nitric oxide

ACE inhibitors improve endothelium-dependent vasodilation in peripheral and coronary arteries, both after short-term and long-term administration.1–5 This beneficial effect, which appears to involve bradykinin and/or the bradykinin B2 receptor,1–5 may contribute to the beneficial long-term effects of ACE inhibitors in arteriosclerotic vascular disease, resulting in reduced mortality and morbidity rates in this patient population.6 In contrast to ACE inhibitors, angiotensin type 1 (AT1) receptor antagonists are thought to act through the AT1 receptor without affecting the breakdown of bradykinin, thereby avoiding the undesirable side effects of ACE inhibitors. However, there is increasing experimental evidence that AT1A can enhance endothelium-dependent relaxation and that this effect is, in part, mediated by bradykinin and nitric oxide.7,8

Endothelium-dependent relaxation of coronary and peripheral arteries, including flow-dependent dilation (FDD), is impaired in patients with coronary artery disease (CAD)2,3,9–11 and may dispose these patients to increased cardiovascular risk. AT1A have become popular drugs for treatment of hypertension and, more recently, for heart failure, but at the same time, the apparent lack of inhibition of the bradykinin breakdown has raised some doubts of equal cardiovascular potency as compared with ACE inhibitors.

This study was designed to test the hypothesis that AT1A improve the impaired endothelium-mediated vasodilation in patients with CAD and to elucidate the contribution of bradykinin and NO. Accordingly, we determined the effect of intra-arterial infusion of the AT1A candesartan on endothelium-mediated vasodilation alone and during coinfusion with N-monomethyl-L-arginine (L-NMMA) to inhibit NO synthesis or icatibant to block the bradykinin B2 receptor.

Methods

The study (with institutional ethics committee approval) was performed in 20 patients with CAD who had given written informed consent. All procedures were in accordance with institutional guidelines. Patients with diabetes, congestive heart failure, uncontrolled hypertension, or prior therapy with ACE inhibitors or AT1-receptor antagonists were excluded. Patients were randomly assigned to 2 groups. In group A (n=10; age, 57±4 years; LDL cholesterol, 148±12 mg/dL), we determined the effect of candesartan alone and during coinfusion with icatibant. In group B (n=10; age, 56±3 years; LDL cholesterol, 163±12 mg/dL), we determined the effect of candesartan alone and during coinfusion with L-NMMA. Radial artery diameter and blood flow was measured and FDD was...
performed as published recently.\textsuperscript{1,12} Arterial blood pressure and heart rate were measured by cuff technique on the contralateral arm.

After insertion of a polyethylene catheter into the brachial artery of the nondominant arm, saline was infused, blood flow velocity was recorded, and radial artery diameter was determined.\textsuperscript{12} Wrist occlusion was performed to determine FDD in response to reactive hyperemia.\textsuperscript{12} After obtaining baseline values for blood flow and diameter again, candesartan (ASTRA; 800 µg/min for 5 minutes) was infused, followed by saline during arterial occlusion and determination of FDD after release of arterial occlusion. Dose selection of candesartan was based on results of dose-finding experiments in 8 patients, demonstrating that this dose caused a robust increase in FDD without affecting systemic hemodynamics.

Next, in group A, icatibant (HOE 140; 90 µg/min for 5 minutes\textsuperscript{1}) was coinfused with candesartan and FDD was determined again. In group B, L-NMMA (7 µmol/min; 5 minutes\textsuperscript{12}) was coinfused with candesartan and FDD was determined again. Finally, sodium-nitroprusside (SNP; 10 µg/min; 5 minutes) was infused to assess endothelium-independent vasodilatation.\textsuperscript{12} To strengthen the principal findings of the present study, we performed additional control experiments: In 5 patients with CAD (control group), vehicle was infused instead of candesartan; FDD was determined during control conditions and was repeated after 5-minute infusion of vehicle (NaCl 0.9%; vehicle 1) and again after a second infusion of vehicle (vehicle 2).

Furthermore, we determined the effect of icatibant and L-NMMA alone in patients with CAD and compared the effect with the effect of candesartan and coinfusions. In 3 patients (control group candesartan/icatibant), we measured FDD during control conditions, after icatibant, again during control conditions, after candesartan, and after coinfusion of candesartan+icatibant. In 3 additional patients with CAD (control group candesartan/L-NMMA), FDD was determined during control conditions, after L-NMMA, again during control conditions, after candesartan, and after coinfusion of candesartan+L-NMMA.

In addition, we determined the effect of candesartan on SNP-induced vasodilation in 7 patients with CAD (control group SNP/candesartan): The effect of SNP (10 µg/min; 5 minutes) was compared with the effect of SNP during coinfusion with candesartan (800 µg/min for 5 minutes).

Blood flow and diameter data reported for control, candesartan, coinfusions, and SNP represent measurements obtained during the last minute of each infusion.

Data are expressed as mean±SEM. Comparisons of >2 measurements within one group of patients were performed by 1-way ANOVA followed by the Student-Newman-Keuls test. A value of \( P<0.05 \) was considered to be statistically significant.

**Results**

After release of wrist occlusion, a significant increase of radial artery diameter was observed representing FDD, defined as percent increase of vessel diameter (Figure). Under resting conditions, neither infusion of candesartan nor coinfusion of candesartan with icatibant or L-NMMA changed radial artery diameter. During flow-stimulated conditions, however, FDD was improved after candesartan in all patients (Figure). In group A, FDD was reduced after coinfusion of candesartan with icatibant; in group B, FDD was reduced after coinfusion of candesartan with L-NMMA (Figure). The effects of candesartan and coinfusions of candesartan with icatibant or L-NMMA were observed to a similar extent in every subject studied. Intra-arterial infusion of SNP increased the diameter of radial artery (group A, 3.10±0.1 to 3.52±0.2; ie, 13.3±1.0%; group B, 3.41±0.1 to 3.84±0.1 mm; ie, 12.7±1.2%; each group \( P<0.01 \) versus baseline).

In the additional control group of patients, FDD during control conditions was 6.7±0.7%; FDD after infusion of vehicle 1 was 6.3±0.5%, and FDD after infusion of vehicle 2 was 6.6±0.5% (\( P=NS \)).

The results of the control group candesartan/icitabant were FDD (control 1), 7.1±0.4%; FDD (icitabant), 5.0±0.6% (\( P<0.05 \) versus control 1); FDD (control 2), 6.6±0.6%; FDD (candesartan), 10.6±0.6% (\( P<0.05 \) versus control 2); FDD (candesartan+icitabant), 4.8±0.4% (\( P<0.05 \) versus FDD after candesartan).

The results of the control group candesartan/L-NMMA were FDD (control 1), 7.2±0.3%; FDD (L-NMMA), 4.5±0.3% (\( P<0.05 \) versus control 1); FDD (control 2), 7.0±0.3%; FDD (candesartan), 9.2±0.4% (\( P<0.05 \) versus control 2); and FDD (candesartan+L-NMMA), 5.3±0.4% (\( P<0.05 \) versus FDD after candesartan). The results of these additional measurements demonstrate that icatibant and L-NMMA (both of which did not affect resting diameter of the brachial artery per se) reduce FDD, suggesting that both bradykinin/B\textsubscript{2} receptor and NO contribute to FDD of the radial artery in patients with CAD. In addition, we show after a second control measurement of FDD that the beneficial effect of candesartan on FDD is reduced by coinfusion with icatibant and L-NMMA down to values after icatibant or L-NMMA alone.

In the control group SNP/candesartan, the vasodilation after SNP alone was 20.0±2.6%; the vasodilation after coinfusion of SNP and candesartan was 19.7±2.7% (\( P=NS \)). Since candesartan did not affect SNP-induced vasodilation, we did not further investigate the effects of L-NMMA or icatibant on SNP-induced vasodilation.

Radial artery blood flow at rest was not affected by infusion of candesartan or coinfusions with icatibant and L-NMMA (group A: control, 40±7; candesartan, 44±6; candesartan+icitabant, 45±5; group B: control, 52±9; cand-
desartan, 52±10; candesartan + L-NMMA, 45±8 mL/min; 
\( p=\text{NS} \). Maximal blood flow during reactive hyperemia after 
release of wrist occlusion was not affected by infusion of 
candesartan and coinfusion of candesartan with icatibant or 
L-NMMA (group A: control, 110±15; candesartan, 124±16;
candesartan + icatibant, 114±11; group B: control, 109±18;
candesartan, 108±11; candesartan + L-NMMA, 109±16 
\( \text{mL/min}; \ p=\text{NS} \). Infusion of SNP increased radial artery 
flow in all groups to a similar extent (group A: 44±7 to 
79±9; group B: 40±5 to 84±4 mL/min; each \( P<0.05 
\) versus control). Systemic blood pressure and heart rate did 
not change during the experimental protocol.

**Discussion**

The salient finding of the present study is that (1) the 
AT\(_1\)-receptor antagonist candesartan improves the impaired 
flow-dependent, endothelium-mediated vasodilation in 
patients with CAD and (2) the beneficial effect of candesartan 
is mediated by bradykinin/B\(_2\) receptor and NO.

Several groups have demonstrated impaired endothelium-
mediated vasodilation in patients with CAD in coronary 
arteries and in the forearm circulation.\(^2,3,9\) In the present 
study, all patients had severely reduced flow-dependent, 
endothelium-mediated vasodilation as compared with normal 
FDD values established in our laboratory (patients with CAD: 
6 to 8%; normal control subjects: 15±1%).\(^1,12\) FDD was 
increased by >40% after local intra-arterial infusion of 
candesartan, demonstrating that AT\(_1\)A improve endothelial 
function in patients with CAD, consistent with previous 
arstings in peripheral artery disease or diabetes.\(^13,14\) The 
beneficial effect of candesartan on vascular function is 
restricted specifically on endothelial function and cannot be 
explained by improved vascular smooth muscle function, 
since SNP-induced vasodilation of the radial artery was 
unaffected by coinfusion with candesartan. This is in line 
with our recent observation that long-term therapy with 
losartant did not affect the effect of intra-arterial SNP on radial 
artery diameter.\(^15\) In contrast, the beneficial effect of cande-
sartan on FDD in our patients with CAD was prevented by 
coinfusion with L-NMMA. In fact, candesartan significantly 
increased the porportion of FDD mediated by NO (represented 
by the porportion of FDD inhibited by L-NMMA), clearly 
indicating that the AT\(_1\)A increase the bioavailability of NO. 
The specificity of this result finds further support by our 
vehicle control experiments demonstrating no change of FDD 
after repeated measurements. Accordingly, the changes of 
FDD after L-NMMA or coinfluosons cannot be explained by 
anunspecific negative effect of repeated determinations of 
FDD but represen specific effects. Furthermore, we 
performed additional experiments including a second control 
measurement of FDD after the end of L-NMMA infusion, 
demonstrating FDD values comparable to baseline conditions 
before L-NMMA. This result further supports our concept of 
specific drug effects with limited duraton after infusion of 
L-NMMA, candesartan, and coinfluosons. Our findings are 
therefore consistent with experimental findings in dog coro-
nary arteries demonstrating that losartan improved endothe-
lum-mediated vasomotion, an effect that was prevented by 
the NO-synthase inhibitor L-NAME, suggesting that this 
effect was mediated by NO.\(^7\) Although short-term improve-
ment of endothelium-dependent relaxation by AT\(_1\)A and the 
involvement of NO have been observed in experimental 
arstings and the present clinical investigation, the 
underlying mechanism(s) mediating this NO-dependent ef-
ef of AT\(_1\)A remained unclear. However, recent findings in 
transgenic mice have delineated the interaction of NO, 
bradykinin, and the angiotensin type 2 (AT\(_2\)) receptor.\(^16\) 
Endothelial cells express the bradykinin B\(_2\) receptor, which, 
when activated, stimulates the production and release of NO. 
In spontaneously hypertensive rats, AT\(_2\) activation has been 
shown to increase vascular cGMP levels, an effect that could 
be inhibited by bradykinin B\(_2\) receptor blockade, by AT\(_2-
) receptor blockade or inhibition of NO synthesis.\(^8\) AT\(_1\)A 
treatment has been shown to be associated with significant 
increases of plasma levels of angiotensin II,\(^17\) which, in the 
fate of AT\(_1\)A blockade, stimulate the AT\(_2\) receptor.\(^8\) It has 
therefore been suggested that the beneficial effect of AT\(_1\)A on 
endothelial function may be explained by stimulation of the 
AT\(_2\) receptor, leading to activation of the bradykinin-NO 
cascade. This concept is supported by recent work of Tsu-
sumi et al,\(^18\) demonstrating that angiotensin II leads to 
vasodilation instead of vasoconstriction in transgenic mice 
overexpressing the AT\(_2\) receptor, an effect that was prevented 
by the bradykinin B\(_2\)-receptor antagonist icatibant and the 
NO-synthase inhibitor L-NAME. In fact, these investigators 
suggest that AT\(_2\)-mediated activation of the Na\(^+\)H\(^{-}\) ex-
changer promotes intracellular acidosis and subsequent acti-
vation of kininogenses that would enhance kinin formation, 
which, in turn, stimulates the release of NO.\(^18\)

The results of our present work are consisten with this 
concept, since the beneficial effect of candesartan on endo-
thelium-mediated vasodilation in response to increased flow 
was prevented by concomitant infusion of the B\(_2\)-receptor 
agonist icatibant. Notably, previous clinical observations 
from our group and experimental findings in bradykinin B\(_2\) 
receptor knockout mice have shown that bradykinin is in-
volved in flow-dependent vasodilation.\(^19,20\) The present study 
extends these observations by showing that (1) the contribu-
tion of endogenous bradykinin to FDD is limited during 
control conditions (represented by the difference: FDD con-
trol minus FDD after icatibant) and that (2) the contribution 
of endogenous bradykinin is significantly increased after 
short-term AT\(_1\)A with candesartan (represented by the differ-
ence FDD after candesartan minus FDD after candesartan+icatibant). Our data support the concept that the 
short-term AT\(_1\)A-mediated, enhanced FDD in vivo in patients 
with CAD is related to a bradykinin/B\(_2\)-receptor dependent 
mechanism.

In the present study, however, it was not possible to 
investigate the contributon of the AT\(_2\)R after treatment with 
candesartan directly, because specific AT\(_2\)-A, such as 
PD123319, are not available for application in humans. 
However, activation of AT\(_2\) receptors by endogenous angio-
tensin II has been shown to be involved in FDD in rat 
resistance arteries.\(^21\)

If the experimental observations and proposed mechanisms 
are operating in humans, the contribution of the bradykinin/B\(_2\) 
receptor to increased FDD after short-term AT\(_1\)A treatment
would be, however, restricted to tissues with sufficient expression of the AT₁ receptor. The involvement of bradykinin after short-term AT₁ does not exclude other mechanisms of AT₁ blockade can contribute to improved endothelial function after prolonged treatment. In this respect, we have recently presented indirect evidence that the beneficial effect of long-term therapy with losartan on endothelial function is, in part, related to antioxidative effects. In patients with CAD, the beneficial effect of an intra-arterial infusion of the antioxidant vitamin C on endothelium-dependent relaxation was lost after 4 weeks of therapy with losartan, probably, in part, related to increased activity of the endothelial-bound superoxide dismutase.¹⁵

In conclusion, our present work has demonstrated that short-term administration of AT₁A enhance endothelial function in patients with CAD, supporting the concept that activation of the bradykinin/NO cascade is involved in the vascular effects of AT₁A in humans.

References
AT₁-Receptor Antagonism Improves Endothelial Function in Coronary Artery Disease by a Bradykinin/B₂-Receptor-Dependent Mechanism
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