AT$_1$-Receptor Antagonism Improves Endothelial Function in Coronary Artery Disease by a Bradykinin/B$_2$-Receptor-Dependent Mechanism

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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 (AT$_1$A) enhance endothelium-dependent relaxation in CAD. The present study was designed to determine whether AT$_1$A 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 B$_2$-receptor antagonist (90 µg/min; group A) or N-monomethyl-L-arginine (L-NMMA), an NO-synthase inhibitor (7 µmol/min; group B). The AT$_1$A 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 AT$_1$A-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. This beneficial effect, which appears to involve bradykinin and/or the bradykinin B$_2$ receptor, 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. In contrast to ACE inhibitors, angiotensin type 1 (AT$_1$) receptor antagonists are thought to act through the AT$_1$ receptor without affecting the breakdown of bradykinin, thereby avoiding the undesirable side effects of ACE inhibitors. However, there is increasing experimental evidence that AT$_1$A can enhance endothelium-dependent relaxation and that this effect is, in part, mediated by bradykinin and nitric oxide.

Endothelium-dependent relaxation of coronary and peripheral arteries, including flow-dependent dilation (FDD), is impaired in patients with coronary artery disease (CAD) and may dispose these patients to increased cardiovascular risk. AT$_1$A 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 AT$_1$A 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 AT$_1$A 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 B$_2$ 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 AT$_1$A-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 measured.
performed as published recently. 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. Wrist occlusion was performed to determine FDD in response to reactive hyperemia. 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) was coinfused with candesartan and FDD was determined again. In group B, L-NMMA (7 μmol/min; 5 minutes) was coinfused with candesartan and FDD was determined again. Finally, sodium-nitroprusside (SNP; 10 μg/min; 5 minutes) was infused to assess endothelium-independent vasodilation. 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/icatibant were FDD (control 1), 7.1±0.4%; FDD (icatibant), 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+icatibant), 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/B1 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+icatibant, 45±5; group B: control, 52±9; can-
desartan, 52±10; candesartan+L-NMMA, 45±8 mL/min; P=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 mL/min; P=NS). Infusion of SNP increased radial artery blood 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 AT1-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/B2 receptor and NO.

Several groups have demonstrated impaired endothelium-mediated vasodilation in patients with CAD in coronary arteries and in the forearm circulation. Several patients who 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%). FDD was increased by >40% after local intra-arterial infusion of candesartan, demonstrating that AT1A improve endothelial function in patients with CAD, consistent with previous observations in peripheral artery disease or diabetes. 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 losartan did not affect the effect of intra-arterial SNP on radial artery diameter. In contrast, the beneficial effect of candesartan on FDD in our patients with CAD was prevented by coinfusion with L-NMMA. In fact, candesartan significantly increased the portion of FDD mediated by NO (represented by the portion of FDD inhibited by L-NMMA), clearly indicating that the AT1A 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 coinusions cannot be explained by an unspecific negative effect of repeated determinations of FDD but represent 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 duration after infusion of L-NMMA, candesartan, and coinusions. Our findings are therefore consistent with experimental findings in dog coronary arteries demonstrating that losartan improved endothelium-mediated vasomotion, an effect that was prevented by the NO-synthase inhibitor L-NAM, suggesting that this effect was mediated by NO. Although short-term improvement of endothelium-dependent relaxation by AT1A and the involvement of NO have been observed in experimental investigations and the present clinical investigation, the underlying mechanism(s) mediating this NO-dependent effect of AT1A remained unclear. However, recent findings in transgenic mice have delineated the interaction of NO, bradykinin, and the angiotensin type 2 (AT2) receptor.16 Endothelial cells express the bradykinin B2 receptor, which, when activated, stimulates the production and release of NO. In spontaneously hypertensive rats, AT2 activation has been shown to increase vascular cGMP levels, an effect that could be inhibited by bradykinin B2 receptor blockade, by AT2 receptor blockade or inhibition of NO synthesis. AT2A treatment has been shown to be associated with significant increases of plasma levels of angiotensin II, which, in the face of AT1A blockade, stimulate the AT2A receptor. It has therefore been suggested that the beneficial effect of AT1A on endothelial function may be explained by stimulation of the AT2A receptor, leading to activation of the bradykinin-NO cascade. This concept is supported by recent work of Tsutsumi et al, demonstrating that angiotensin II leads to vasodilation instead of vasoconstriction in transgenic mice overexpressing the AT2 receptor, an effect that was prevented by the bradykinin B2-receptor antagonist icatibant and the NO-synthase inhibitor L-NAME. In fact, these investigators suggest that AT2-mediated activation of the Na+/H+ exchanger promotes intracellular acidosis and subsequent activation of kinogenases that would enhance kinin formation, which, in turn, stimulates the release of NO.

The results of our present work are consistent with this concept, since the beneficial effect of candesartan on endothelium-mediated vasodilation in response to increased flow was prevented by concomitant infusion of the B2-receptor antagonist icatibant. Notably, previous clinical observations from our group and experimental findings in bradykinin B2 receptor knockout mice have shown that bradykinin is involved in flow-dependent vasodilation. The present study extends these observations by showing that (1) the contribution of endogenous bradykinin to FDD is limited during control conditions (represented by the difference: FDD control minus FDD after icatibant) and that (2) the contribution of endogenous bradykinin is significantly increased after short-term AT1A with candesartan (represented by the difference FDD after candesartan minus FDD after candesartan+icatibant). Our data support the concept that the short-term AT1A-mediated, enhanced FDD in vivo in patients with CAD is related to a bradykinin/B2-receptor dependent mechanism.

In the present study, however, it was not possible to investigate the contribution of the AT2R after treatment with candesartan directly, because specific AT2A, such as PD123319, are not available for application in humans. However, activation of AT2 receptors by endogenous angiotensin 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/B2 receptor to increased FDD after short-term AT1A treatment
would be, however, restricted to tissues with sufficient expression of the AT2 receptor. The involvement of bradykinin after short-term AT2 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

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