Vascular Response to Angiotensin II Predicts Long-Term Prognosis in Patients Undergoing Coronary Artery Bypass Grafting

Pim van der Harst, Meint Volbeda, Adriaan A. Voors, Hendrik Buikema, Sven Wassmann, Michael Böhm, Georg Nickenig, Wiek H. van Gilst

Abstract—Persistent activation of the renin-angiotensin system leads to downregulation of the angiotensin type-1 receptor, and consequently, to a decreased response to exogenous angiotensin II. In the present study, we investigated the association of angiotensin II responsiveness to clinical outcome after coronary artery bypass grafting (CABG). We studied the responsiveness to exogenous angiotensin II in human thoracic artery preparations of 114 CABG patients. Mean duration of follow-up was 7.3 ± 0.1 years, during which 21 patients experienced a cardiovascular event. A diminished response to angiotensin II remained in multivariate Cox regression analysis, after adjustment for sex, age, blood pressure, and number of diseased coronary arteries, the strongest predictor for cardiovascular events (relative risk, 3.37 [95% confidence interval, 1.20 to 9.51]; P = 0.022). Furthermore, diminished response to angiotensin II was associated with an increased mean arterial pressure (102.85 ± 1.38 versus 97.40 ± 1.37; P = 0.003) and a nonsignificant increase in angiotensin-converting enzyme activity, suggestive for a persistently activated renin-angiotensin system. In conclusion, these results suggest that in patients undergoing CABG, a diminished vascular responsiveness of the thoracic artery to exogenous angiotensin II is related to an increased risk of future cardiovascular events. (Hypertension. 2004;44:930-934.)

Key Words: angiotensin II ■ resistance ■ vasoconstriction ■ risk factors ■ coronary artery disease

An activated renin-angiotensin system (RAS) is characterized by increased serum levels of angiotensin II (Ang II) and is involved in hypertension, coronary heart disease, heart failure, and other cardiovascular diseases. Increased plasma levels of Ang II, despite angiotensin-converting enzyme (ACE) inhibition, is associated with increased mortality. Although the knowledge of the pathophysiological role of the RAS continues to increase, and treatment with ACE inhibitors and angiotensin receptor blockers (ARBs) are well proven and accepted among clinicians worldwide, prognostic studies of direct assessment of vascular responsiveness to Ang II are lacking. Local or circulating Ang II can decrease the expression or the angiotensin type 1 (AT1) receptor.2-5 This agonist-induced downregulation can subsequently result in relative resistance to Ang II.2,3,6 It remains to be determined whether the response of the human vasculature to Ang II in patients with coronary heart disease can be related to an activated RAS and whether it is associated with future cardiovascular events. We undertook the present study to investigate whether or not the vascular responsiveness to Ang II in thoracic arteries from patients undergoing coronary artery bypass grafting (CABG) predicted long-term outcome for cardiovascular events, including death, myocardial infarction, stroke, and vascular surgery. In this article, we present the prognostic significance of 7.3 years of follow-up. Furthermore, we assessed the relation of vascular responsiveness to Ang II with blood pressure and ACE activity as indicators of an activated RAS.

Methods

Study Population

Patients in the present study participated in the QUinapril On Vascular Ace and Determinants of Ischemia Study (QUO VADIS). A total of 187 patients were included into QUO VADIS, and methods and results of this study were published previously.7,8 We studied all 114 subjects undergoing CABG, for whom Ang II responsiveness was assessed. The institutional review board approved this study, and written informed consent was obtained from each subject.

In Vitro Vascular Measurements

Measurement of vascular responsiveness to Ang II of human internal thoracic arteries performed in the QUO VADIS study has been described previously.9 In brief, excess graft material as obtained during CABG was cut into several 2-mm rings and mounted in...
ACE Activity Determination
Plasma ACE activity was measured 1 day before CABG as described previously. Briefly, using 35\textsuperscript{m} diluted plasma, 10 minutes of incubation with 7 mmol/L hippuryl-L-histidyl-L-leucine (Hip-His-Leu) at 37°C, the production of hippuric acid (nmol/L Hip-Leu per minute per mL) was measured spectrophotometrically. Local ACE activity was determined in arbitrary units as the area between the Ang I and II dose-response curves, normalized for maximal response to Ang II, as described previously.

Long-Term Follow-Up
Long-term follow-up was performed by telephone contact. All cardiovascular events were validated by review of medical records. The outcome measure assessed was the time from CABG until the first occurrence of a component of the following: cardiovascular death, hospitalization for myocardial infarction, revascularization with percutaneous coronary intervention (PCI) or re-CABG (if these procedures were performed at least 30 days after randomization), vascular surgery, and stroke.

Statistical Analysis
Data are expressed as mean±SEM. Statistical significance of differences in baseline characteristics was assessed by unpaired Student \( t \) test or \( \chi^2 \) test when appropriate. Cardiovascular event rates were estimated by Kaplan–Meier survival curves and were compared by means of the log-rank method. Cox proportional hazards multivariate stepwise regression analysis was used to determine the multivariate relationships between clinical variables and cardiovascular events during the follow-up period. Covariates entered in this regression model were Ang II resistance, number of diseased vessels, age, sex, smoking, hypertension, blood pressure, and LDL-cholesterol. Statistical analysis was performed with SPSS statistical software (SPSS). All \( P \) values were 2-tailed, and a value \(<0.05\) was considered to indicate statistical significance.

<table>
<thead>
<tr>
<th>Table 1. Index of Cardiovascular Events During Follow-Up</th>
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<tbody>
<tr>
<td><strong>Outcome</strong></td>
</tr>
<tr>
<td>Cardiac death</td>
</tr>
<tr>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>PCI</td>
</tr>
<tr>
<td>Re-CABG</td>
</tr>
<tr>
<td>Stroke</td>
</tr>
<tr>
<td>Vascular surgery</td>
</tr>
<tr>
<td>Total events</td>
</tr>
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</table>

\( P \) value for low vs high responsiveness to Ang II.

### Results
Of the 114 patients, 1 patient was lost to follow-up. Mean duration of follow-up was 7.3±0.12 years (median 7.4 years). During this period, 21 (18\%) experienced a cardiovascular event, and 2 of these subjects had 2 events (Table 1). Baseline characteristics are presented in Table 2. Their average age at baseline was 62.5±8.3 years, and 16 (14\%) were women. Before CABG, 40\% of patients had had a myocardial infarction, and 15\% had previously undergone a PCI. At baseline, the total cholesterol level was 6.2±1.3 mmol/L (241 mg/dL), LDL-cholesterol was 4.2±1.0 mmol/L (164 mg/dL), HDL-cholesterol was 1.09±0.31 mmol/L (42 mg/dL), and triglycerides 1.96±1.25 mmol/L (174 mg/dL).

Median contraction to Ang II of the total population was 58.4\% of the maximal response to phenylephrine. Maximal contraction to Ang II in the low-responsive group was 38±2...
was the most significant predictor for cardiovascular events (relative risk, 3.37 [95% confidence interval {CI}, 1.20 to 9.50]; \( P = 0.022 \), Table 3). When only the more objective events (cardiac death, myocardial infarction, and stroke) were combined, Ang II remained the most significant predictor (relative risk, 5.0 [95% CI, 1.06 to 23.62]). Response to phenylephrine was not associated with cardiovascular events. Among the individual components of the primary end point, there was a consistent pattern of benefit favoring the high-responsive Ang II over the low-responsive Ang II group, which included a significant association with fewer strokes (\( P = 0.006 \); Table 1).

**Discussion**

We are the first to report long-term follow-up data on in vitro assessment of vasomotor function. The present study demonstrated that in vitro resistance to exogenous Ang II independently predicts the long-term risk of cardiovascular events, including cardiac death, myocardial infarction, PCI, re-CABG, stroke, and vascular surgery after adjustment for blood pressure and other cardiac risk factors. A total of 78% of all cardiovascular events occurred in patients in the Ang II–resistant group. In addition, Ang II resistance was associated with a higher blood pressure and a trend to increased serum and local ACE activity.

Ang II is the principal mediator of the RAS. The AT\(_1\) receptor mediates many of the known detrimental effects of Ang II, including vasoconstriction. Increased levels of endogenous Ang II have been associated with an increased mortality in chronic heart failure (CHF) patients.\(^\text{1}\) Progression of CHF is associated with a progressive increase in cardiac Ang II formation, regardless of etiology of CHF.\(^\text{11}\) More important, in contrast to nonfailing myocytes, myocytes from CHF patients are relatively resistant to Ang II because they are selectively unable to produce appreciable amounts of insulin-like growth factor 1 and endothelin 1 in response to Ang II stimulation.\(^\text{11}\) In CHF, downregulation of AT\(_1\) receptor has been reported by several studies.\(^\text{12–14}\) Increased levels of endogenous Ang II itself diminish AT\(_1\) receptor expression.\(^\text{3–5}\) In our study, we demonstrated diminished responsiveness to Ang II, which might be explained as a reflection of chronic overactivity of the RAS. This is supported by the increased blood pressure and trend to increased local and serum ACE activity.

Despite chronic ACE inhibitor therapy, conversion of Ang I to Ang II may persist, and Ang II levels may return to pretreatment levels.\(^\text{15,16}\) Non-ACE–dependent pathways, such as chymases,\(^\text{17–19}\) are thought to be involved. We assume that the observed diminished responsiveness to exogenous Ang II, relative resistance, in our population is attributable to downregulation of the vascular AT\(_1\) receptor and can be explained by an increase in vivo endogenous Ang II levels. One could also speculate on changes in signaling of AT\(_1\) receptor or differences in AT\(_2\) receptor function. Nevertheless, effects mediated by eNOS by AT\(_2\) receptor or otherwise are not likely because vascular measurements were performed in the continuous presence of the eNOS inhibitor l-NMMA.
The observed difference in stroke between groups is intriguing. Although blood pressure levels were different, our multivariate analysis suggests the Ang II responsiveness to be of greater importance. This suggestion is in good harmony with the Losartan Intervention For End point reduction (LIFE) study, which demonstrated that the ARB losartan substantially reduced the rate of stroke, over and above blood pressure–lowering therapy.20,21 Potential interactions between medical treatment and Ang II response in the current study cannot be excluded. Nevertheless, in our opinion, it is not feasible to obtain less confounded clinical data considering the current clinical guidelines.22,23 Any future study would include even more confounding drug therapy regimes to make it unfeasible to assess the consequence of Ang II resistance.

Of note, we examined internal thoracic arteries. The response of these vessels does not represent the blood pressure increase as measured in humans because blood pressure increases are more dependent of resistance vessels. The local RAS might be differently regulated and is likely to be completely independent of the circulating system. Furthermore, we only assessed the internal thoracic artery; but it is tempting to speculate AT1 receptor upregulation in atherosclerotic coronary or cerebral arteries and downregulation of AT1 receptors in internal thoracic arteries in our patient population. The systemic or local increased Ang II levels might therefore be deleterious in the coronary and cerebral artery. The macroscopic nonatherosclerotic internal thoracic artery might have downregulated the AT1 receptor rather than upregulated it because of the increased circulating in vivo Ang II level.

Study Limitations
This study was descriptive in nature, conducted retrospectively, and will therefore require confirmation in a prospective investigation. Our results cannot be extended to conditions other than coronary artery disease because we included only patients who underwent CABG. We were unable to rule out other factors (eg, medication) known to influence the RAS and AT1 receptor expression. However, medical treatment was similar in both groups. Because serum Ang II levels do not reflect local Ang II levels, the local ACE activity was assessed. Nevertheless, the present data are unique in their kind and provide important pathophysiological insight. We are not aware of a larger series of vascular assessment of Ang II responsiveness performed within a single structured protocol, and the degree of Ang II resistance is an important predictor of outcome in these patients.

Perspectives
We have demonstrated a strong association between Ang II resistance of the human thoracic artery and an adverse long-term cardiovascular prognosis. Although clinical value may be limited, assessment of Ang II resistance may provide a surrogate end point to evaluate therapy. Whether strategies that improve Ang II responsiveness will uniformly improve prognosis needs to be studied prospectively.

Acknowledgments
P.v.d.H. is supported by Zon-MW 920-03-236.

References


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Hypertension. 2004;44:930-934; originally published online October 25, 2004;
doi: 10.1161/01.HYP.0000147823.50497.a9

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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