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.1 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.7 In brief, excess graft material as obtained during CABG was cut into several 2-mm rings and mounted in...
15-mL organ chambers. Measurements of in vitro vascular function took place within 3 hours after harvesting. Rings were connected to an isotonic displacement transducer, in which a preload of 1.4 g was given. Rings were allowed to equilibrate for 1 hour. All rings were primed and checked for viability by repeated stimulation with 10 μmol/L phenylephrine. The responses to Ang I and II (0.1 mmol/L to 1 μmol/L) were studied in parallel rings under continuous presence of Nω-monomethyl-L-arginine (l-NMMA; 100 μmol/L) to avoid confounding NO release by endothelial NO synthase (eNOS). At the end of the angiotensin measurements, a control response was evoked with 10 μmol/L phenylephrine. Results are presented as percentage of the maximal phenylephrine-induced response. To study the relationship between Ang II responsiveness and cardiovascular events, the study population was divided according to the median of maximal response to Ang II, as decided before conducting follow-up. The lower half was considered to have relative resistance to Ang II (in parallel with relative resistance to insulin in diabetes mellitus).

ACE Activity Determination
Plasma ACE activity was measured 1 day before CABG as described previously. Briefly, using 35% 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 His-Leu per mol/L) to 1/200 was studied in parallel rings under continuous presence of Ang I and II dose-response curves, normalized for maximal response activity was determined in arbitrary units as the area between the

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

Statistical Analysis
Data are expressed as mean±SEM. Statistical significance of differences in baseline characteristics was assessed by unpaired Student t test or χ² 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>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome</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>
</tbody>
</table>

P value for low vs high responsiveness to Ang II.

<table>
<thead>
<tr>
<th>TABLE 2. Clinical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Gender, male/female</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Never smoked</td>
</tr>
<tr>
<td>Current smokers</td>
</tr>
<tr>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>1-vessel disease</td>
</tr>
<tr>
<td>2-vessel disease</td>
</tr>
<tr>
<td>3-vessel disease</td>
</tr>
<tr>
<td>Ischemic NYHA class (1–2/3–4)</td>
</tr>
<tr>
<td>History, n (%)</td>
</tr>
<tr>
<td>Angina (past/current)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>PTCA</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
</tr>
<tr>
<td>Systolic</td>
</tr>
<tr>
<td>Diastolic</td>
</tr>
<tr>
<td>Lipid concentrations, mmol/L</td>
</tr>
<tr>
<td>Total cholesterol</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
</tr>
<tr>
<td>Triglycerides</td>
</tr>
<tr>
<td>Medication (%)</td>
</tr>
<tr>
<td>Quinapril/captopril/placebo</td>
</tr>
<tr>
<td>β-blocker</td>
</tr>
<tr>
<td>Nitrates</td>
</tr>
<tr>
<td>Lipid lowering</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association; PTCA, percutaneous transluminal coronary angioplasty.

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...
percent phenylephrine) and in the high-responsive group
74/2 (Figure 1). Absolute contractions for phenylephrine
was 346/23 and 444/34 m (28% difference; \( P = 0.02 \)) and
for Ang II 135/12 and 323/26 m (139% difference;
\( P < 0.00001 \)) in the low- and high-responsive group,
respectively. Low responsiveness to Ang II was associated with an
increased mean arterial blood pressure (102.9/1.4 versus
97.4/1.4; \( P = 0.003 \)). Serum ACE activity (23.7/1.2 nmol/L
His-Leu per minute per mL versus 18.3/2.7; \( P = 0.12 \))
and local ACE activity (17.5/0.7 arbitrary units versus
16.3/0.5; \( P = 0.19 \)) were not significantly increased in pa-
tients with low responsiveness to Ang II.

For all patients, the Kaplan–Meier event rates of the
primary end point was 29% in the low-responsive group and
9% in the high-responsive group. The high-responsive group
was associated with 69% fewer events (\( P = 0.0059 \); Figure 2).

In multivariate Cox regression analysis, after adjustment for
sex, age, mean arterial blood pressure, and number of
diseased coronary arteries, a diminished response to Ang II
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-
resistant 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 demon-
strated that in vitro resistance to exogenous Ang II indepen-
dently 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 associ-
ated with a higher blood pressure and a trend to an increased
serum and local ACE activity.

Ang II is the principal mediator of the RAS. The AT1
receptor mediates many of the known detrimental effects of
Ang II, including vasoconstriction. Increased levels of endog-
ogenous Ang II have been associated with an increased mortal-
ity in chronic heart failure (CHF) patients.1 Progression of
CHF is associated with a progressive increase in cardiac Ang
II formation, regardless of etiology of CHF.1 More impor-
tant, 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.11 In CHF, downregulation of AT1 receptor has
been reported by several studies.12–14 Increased levels of
dogenous Ang II itself diminish AT1 receptor expres-
sion.5–7 In our study, we demonstrated diminished respon-
siveness 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.15,16 Non-ACE–dependent pathways,
such as chymases,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 AT1 receptor and can be
explained by an increase in vivo endogenous Ang II levels.
One could also speculate on changes in signaling of AT1
receptor or differences in AT2 receptor function. Neverthe-
less, effects mediated by eNOS by AT2 receptor or otherwise
are not likely because vascular measurements were per-
formed in the continuous presence of the eNOS inhibitor
l-NMMA.

Figure 1. Concentration-response curves of Ang II–induced
vasoconstriction.

Figure 2. Kaplan–Meier curve for primary composite end point.
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.

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**References**


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