Angiotensin-Converting Enzyme Inhibition Alters the Fibrinolytic Response to Cardiopulmonary Bypass

Mias Pretorius, MBChB, MSc; Laine J. Murphey, MD, PhD; Julie A. McFarlane, RN; Douglas E. Vaughan, MD; Nancy J. Brown, MD

Background—Increased plasminogen activator inhibitor-1 (PAI-1) concentrations after coronary artery bypass grafting (CABG) are associated with increased risk of vein graft occlusion. Because angiotensin II stimulates PAI-1 expression, we tested the hypothesis that preoperative angiotensin-converting enzyme (ACE) inhibition decreases PAI-1 expression after CABG.

Methods and Results—We measured the effects of cardiopulmonary bypass (CPB) on PAI-1 antigen and tissue-type plasminogen activator (tPA) antigen and activity in 31 patients taking an ACE inhibitor (ACEI) who were randomized to continue ACEI until the morning of surgery (ACEI group, n = 19) or to discontinue it 48 hours before surgery (No-ACEI group, n = 12). Arterial blood samples were taken at baseline before CPB, twice during CPB, after separation from CPB, and on postoperative day 1 (POD1). CPB caused an early decrease in PAI-1 antigen, followed by an increase in PAI-1 antigen on POD1 (P < 0.001 for effect of time). ACE inhibition attenuated the increase in PAI-1 antigen such that both PAI-1 antigen on POD1 (P = 0.013) and the change in PAI-1 antigen from baseline to POD1 (P = 0.009) were higher in the No-ACEI group (17.0 ± 5.0 to 48.7 ± 8.8 ng/mL) versus the ACEI group (from 19.9 ± 3.4 to 33.1 ± 6.2 ng/mL). There was no significant difference between the 2 groups in intraoperative tPA activity (P = 0.259); however, the increase in tPA activity was significantly greater in the ACEI group than in the No-ACEI group (P = 0.030).

Conclusions—Preoperative ACEI attenuates the increase in PAI-1 after CABG, suggesting a role for ACE inhibition in reducing the risk of acute graft thrombosis. (Circulation. 2003;108:3079-3083.)

Key Words: plasminogen activators □ inhibitors □ angiotensin □ cardiopulmonary bypass □ thrombosis

A cute saphenous vein graft occlusion occurs in 12% to 23% of patients undergoing CABG,1,2 causing significant morbidity and mortality. Alterations in the vessel wall, changes in blood rheology, and altered flow dynamics can all contribute to graft thrombosis in the postoperative period. In addition, cardiopulmonary bypass (CPB) causes inflammation, leading to an increase in plasminogen activator inhibitor-1 (PAI-1) in the early postoperative period.3 PAI-1 inhibits fibrinolysis by forming a complex with tissue plasminogen activator (tPA), thereby preventing its interaction with plasminogen. Significantly, several studies3-4 indicate that increased PAI-1 antigen or activity in the perioperative period is associated with early vein graft occlusion after CABG.

Angiotensin-converting enzyme (ACE) inhibitors (ACEIs) decrease the formation of angiotensin II, a potent stimulus of PAI-1 expression,5 and decrease the degradation of bradykinin, a potent stimulus of endothelial tPA release.6 ACEIs decrease circulating PAI-1 concentrations in salt-depleted normal subjects,7,8 in hypertensive individuals,9 in postmenopausal women,10 and in patients after myocardial infarction.11 Recently, Wagner et al12 reported that ACEIs decrease PAI-1 antigen after thrombolysis. The purpose of this study was to test the hypothesis that preoperative ACE inhibition decreases PAI-1 antigen concentration in patients undergoing CABG.

Methods

Patients
Thirty-one patients were studied. Patients were eligible if they were undergoing elective CABG and were taking an ACEI preoperatively. Patients were excluded if they had evidence of coagulopathy (INR > 1.7), underwent emergency surgery, underwent redo CABG, had taken a glycoprotein IIb/IIIa antagonist within 2 days of surgery, or had renal insufficiency (baseline creatinine > 1.5 mg/dL). Eligible patients were randomized to continue ACEI until the morning of surgery (ACEI group, n = 19) or to discontinue it ≥ 48 hours before surgery (No-ACEI group, n = 12). Randomization was performed according to a permuted block randomization scheme with a block size, or balancing interval, varying randomly between 4 and 6 according to the outcome of a random-number table. All patients provided written informed consent.

Protocol
The study protocol was approved by the Vanderbilt University and VA Tennessee Valley Healthcare System Institutional Review Boards and conducted according to the Declaration of Helsinki. Anesthesia management and CPB were conducted according to institutional protocol. Induction of anesthesia was achieved with...
either etomidate or thiopental and maintained with isoflurane, fentanyl, air, and oxygen. Muscle relaxation was achieved and maintained with pancuronium or vecuronium. Hemodynamics were monitored invasively with an arterial line (Arrow International), and a pulmonary artery catheter (Edwards Lifesciences) was placed in patients with a left ventricular ejection fraction <40%. CPB was achieved with a roller or centrifugal pump (Medtronic), a heparin-coated circuit (Carmeda), and a Trillium hollow-fiber oxygenator (Medtronic). Heparin was used for anticoagulation during CPB at an initial dose of 400 U/kg supplemented with additional heparin to achieve and maintain an activated clotting time of >400 seconds. Heparin was neutralized with protamine sulfate after separation from CPB. Moderate systemic hypothermia and cold retrograde and antegrade cardioplegia solution was applied to all patients. The use of aprotinin and e-aminocaproic acid was excluded during this study. After postoperative day 1 (POD1), patients were restarted on ACEIs at the discretion of the cardiothoracic surgeon.

Patients were transfused according to the following guidelines: packed red blood cells were transfused for a hematocrit <20% during CPB and for a hematocrit <25% after CPB, CPB time >120 minutes, or evidence of end-organ dysfunction. Platelets were transfused in 5-U sets for ongoing microvascular bleeding despite a normalized activated clotting time. Fresh frozen plasma was given for continued bleeding only after platelets were given. Transfusion requirements were recorded from the beginning of surgery until hospital discharge.

Although the study was designed only to look at biochemical markers (PAI-1 and tPA), we also collected clinical outcome data for the early postoperative period. The data collected included new-onset dysrhythmias, evidence of myocardial ischemia (ECG changes), and 30-day mortality as possible markers for early vein graft occlusion. Myocardial infarction was defined as ECG evidence of a new pathological Q wave. Episodes of myocardial ischemia were defined as ST-segment changes involving a shift from baseline of ≥1 mm.

**Blood Sampling and Biochemical Assays**

Blood samples were obtained for measurement of hematocrit, ACE activity, PAI-1, and tPA antigen and activity. Samples were collected at 5 time points: (1) after induction of anesthesia and before CPB (baseline); this occurred between 8 AM and 9 AM; (2) at 30 minutes of CPB; (3) at 60 minutes of CPB; (4) after separation from CPB and administration of protamine; and (5) at 9 AM on POD1. All blood samples were taken from an indwelling arterial line.

**PAI-1 and tPA Antigen and Activity**

All blood samples were collected on ice and centrifuged immediately at 0°C for 20 minutes. Plasma was then separated and stored at −70°C until the time of assay. Blood for PAI-1 and tPA assays was collected in Vacutainer tubes containing acidified 0.105 mol/L sodium citrate (Biopool). PAI-1 and tPA antigen levels were determined with a 2-site ELISA (Immulyse, Biopool). tPA activity levels were measured with a chromogenic substrate and a standardized commercial kit (Chromolyse, Biopool), with results expressed as U/mL.

**Plasma ACE Activity**

Serum ACE activity was determined by a 3-step colorimetric assay in which ACE hydrolyzes the substrate p-hydroxybenzoyl-glycyl-l-histidyl-l-leucine, and subsequent reactions led to the formation of quinonimine dye, which was measured spectrophotometrically (Fujirebio America Inc).

**Statistical Analysis**

Data are presented as mean±SEM. Categorical data were compared between groups using χ² or Fisher’s exact tests, as appropriate. Continuous baseline data were compared by Student’s t test or Mann–Whitney U test, as appropriate. Comparisons of the hemodynamic and fibrinolytic responses to CPB between groups were made with a general linear model repeated-measures ANOVA in which the within-subject variable was time and the between-subject variables were ACE inhibition status, diabetes status (diabetes or not), and/or smoking status (active or not). Analyses were repeated separately in individuals who had been treated with low-dose lisinopril (<10 mg/d) versus those treated with usual- to high-dose lisinopril (>10 mg/d). A 2-tailed probability value <0.05 was considered statistically significant. All analyses were performed with the statistical package SPSS for Windows (Version 11.0.1).

### Results

**Subject Characteristics**

Before randomization, 28 patients (17 of 19 in the ACEI group and 11 of 12 in the No-ACEI group) were taking lisinopril in dosages that ranged from 5 to 40 mg/d, 2 patients (1 in each group) were taking captopril 12.5 to 50 mg 3 times a day, and 1 patient (ACEI group) was taking fosinopril 20 mg once a day. Table 1 provides the baseline patient characteristics. There were no significant differences between the 2 groups with regard to age, sex, race, body mass index, preoperative mean arterial pressure (MAP), heart rate, hematocrit, platelet count, preoperative tPA antigen, tPA activity, PAI-1 antigen, smoking status, history of diabetes mellitus, history of hypertension, history of hyperlipidemia, aspirin use, calcium channel blocker use, or β-blocker use. Preoperative left ventricular ejection fraction was significantly higher in the No-ACEI group than in the ACEI group (P=0.040). Preoperative ACE activity was significantly suppressed in the ACEI group compared with the No-ACEI group (P=0.006).

### Hemodynamic Response to CPB

Table 2 provides the intraoperative and postoperative characteristics of the patients. There were no significant differences between the 2 groups with regard to CPB time, cross-clamp time, number of grafts, chest tube output at 4 and...
24 hours, and time to extubation >10 hours. Temperature at 30 minutes of CPB was significantly lower in the No-ACEI group than the ACEI group. MAP decreased significantly during CPB in both the ACEI (from 87.1±3.0 to 58.8±2.7 mm Hg at 60 minutes of CPB; F=27.813, P<0.001) and No-ACEI (from 91.1±2.7 to 65.5±3.7 mm Hg at 60 minutes of CPB; F=17.583, P=0.001) groups; however, MAP was not significantly different between the 2 groups (F=1.952, P=0.174). There were no significant differences in the use of any vasopressor (all P>0.141) between the 2 groups.

Fibrinolytic Response to CPB

Figures 1 to 3 show the effect of ACE inhibition on fibrinolytic balance during and after CPB. CPB elicited a biphasic change in PAI-1 antigen such that PAI-1 antigen concentrations decreased initially during CPB but then increased after CPB (effect of time, F=9.158, P<0.001). The decrease in PAI-1 antigen during CPB was similar in the ACEI and No-ACEI groups (F=0.001, P=0.973 for effect of treatment group). In contrast, the increase in PAI-1 antigen after CPB was blunted in the ACEI group (F=8.316, P=0.009 for time×treatment group interaction). Thus, PAI-1 antigen was significantly increased on POD1 compared with baseline in the No-ACEI group (from 17±5.0 to 48.7±8.8 ng/mL, P=0.004) but not in the ACEI group (from 19.9±3.4 to 33.1±6.2 ng/mL). Both POD1 PAI-1 antigen (P=0.013) and the increase in PAI-1 antigen from baseline to POD1 (P=0.009) were higher in the No-ACEI group than in the ACEI group. Because ACE inhibition has been shown to reduce PAI-1 in a dose-dependent manner,13 we analyzed the effect of lisinopril on PAI-1 separately in individuals who were treated with low doses versus those treated with usual to high doses (Table 3). ACE activity was significantly higher in the low-dose lisinopril versus the usual- to high-dose patients (P=0.028). In the usual- to high-dose lisinopril patients, both POD1 PAI-1 antigen (P=0.011) and the change in PAI-1 antigen from baseline to POD1 (P=0.002) were higher in the No-ACEI group than in the ACEI group.

tPA antigen increased significantly by 60 minutes in the ACEI group only and was higher at 30 and 60 minutes in the ACEI versus the No-ACEI group. The early increase in tPA antigen was greater in the ACEI group than in the No-ACEI group (F=5.193, P=0.031 for effect of treatment).

CPB elicited a biphasic change in tPA activity such that tPA activity concentrations increased initially during CPB with a peak after protamine administration but then decreased after CPB (effect of time, F=45.916, P<0.001). There was no significant difference between the 2 groups in intraoperative tPA activity (P=0.259); however, the increase in tPA activity was significantly greater in the ACEI group than in the No-ACEI group (F=5.282, P=0.030 for effect of treatment).

Transfusion Requirements and Clinical Outcome

Because alterations in fibrinolytic balance could affect requirements for blood product transfusion, we compared transfusion requirements between the 2 groups. There were no significant differences in the total units of packed red blood cells (P=0.124), fresh frozen plasma (P=0.126), or platelets (P=1.0) transfused between the No-ACEI and the ACEI groups. Table 4 provides clinical outcome data. There were no significant differences in new-onset dysrhythmias, myocardial ischemic events, 30-day mortality, or length of hospitalization between the No-ACEI and the ACEI group.

Discussion

This study examined the effect of preoperative ACE inhibition on the fibrinolytic response in patients undergoing...
CABG requiring CPB. Numerous investigators have reported an increase in tPA during CPB followed by an increase in PAI-1 postoperatively.3,14–17 Moor et al4 showed that the increase in PAI-1 on POD1 predicted early vein graft occlusion. The present study demonstrates that preoperative ACE inhibition attenuates the increase in PAI-1 after CABG.

PAI-1 plays a critical role in the regulation of fibrinolysis, serving as the primary inhibitor of tPA. Elevated levels of PAI-1 not only are a risk factor for recurrent myocardial infarction18 but also predict early vein graft occlusion. The present study does not specifically address the mechanism for this increase.5, and also by reducing the enzymatic degradation of bradykinin, a potent stimulus of tPA release.19,20 ACEIs have been shown to reduce circulating PAI-1 antigen in patients with risk factors for coronary artery disease9,13,21 or after myocardial infarction.11,22 In addition, ACE inhibition prevents the increase in PAI-1 after thrombolysis.12

In addition to attenuating the postoperative increase in PAI-1 antigen, ACE inhibition was associated with an enhanced early tPA response to CPB. Although the present study does not specifically address the mechanism for this effect, the increased tPA response to CPB during ACE inhibition may have resulted from decreased bradykinin clearance. CPB stimulates the kallikrein-kinin system, increasing bradykinin concentrations 10- to 20-fold.23,24 Because bradykinin stimulates endothelial tPA release,19,20 decreased bradykinin clearance during ACE inhibition would be expected to increase tPA. Thus, the net effect of ACE inhibition in patients undergoing elective CABG requiring CPB was to increase fibrinolytic activity by decreasing PAI-1 antigen and increasing tPA antigen.

There was no effect of ACE inhibition on MAP or vasoconstrictor requirements during the study. This contrasts with data from several other studies that indicate that ACE inhibition decreases blood pressure25 or increases vasoconstrictor requirement during and after CPB26,27 but not with data from others.28 The lack of apparent effect of ACE inhibition on MAP and vasoconstrictor requirements may relate to the short duration ACEI was stopped in the No-ACEI group. In fact, 1 limitation of this study was the short duration for which the ACEI was discontinued (48 hours) preoperatively in the No-ACEI group. Although ACE activity returned to low normal within 48 hours in the No-ACEI group, tissue ACE activity can remain depressed for a prolonged time after discontinuation of ACE inhibition.29 In this regard, the present study would have tended to underestimate the effect of continued ACE inhibition on blood pressure and fibrinolytic balance.

This study was not designed to assess early vein graft occlusion. For ethical reasons, patients were put back on their ACEI during the postoperative period. Nevertheless, we did collect clinical outcome data relevant to early vein graft occlusion. There were no significant differences in the frequency of new-onset dysrhythmias, myocardial ischemic events, length of hospitalization, or 30-day mortality between the ACEI and No-ACEI groups.

There was also no significant difference in the transfusion requirements between the 2 groups, even though the increase in tPA activity was higher in the ACEI group. In fact, the median transfusion of packed red blood cells and platelets tended to be higher in the No-ACEI group. This may reflect the fact that not only fibrinolysis but also other factors, including platelet dysfunction, age, sex, preoperative hematocrit, duration of CPB, lower-body surface area, lower temperature on bypass, and factor V Leiden, determine postoperative blood product transfusion risk.30–32 For example, in the present study, a significantly lower temperature in the No-ACEI group during CPB may have influenced transfusion requirements. Thus, it would seem that ACE inhibition improves fibrinolytic balance after CPB without increasing bleeding risk.

**Study Limitations**

Although patients were randomized to preoperative ACE inhibition, the present study was neither blinded nor placebo-controlled. In addition, the dose and class of ACEI were not standardized. Nevertheless, all but 3 patients were taking lisinopril. Moreover, previously published reports that enalapril,33 quinapril,8 and ramipril9 (carboxyl-containing ACEIs like lisinopril), fosinopril11 (a phosphinyl-containing ACEI), and captopril14 (a sulphydryl-containing ACEI) all decrease PAI-1 concentrations suggest that the effect of ACE inhibition on PAI-1 is a class effect. The finding that lisinopril decreased postoperative PAI-1 in the present study confirms

---

**TABLE 3. Effect of Lisinopril Dose on PAI-1 Antigen**

| PAI-1 antigen (ng/mL) | ACEI | No ACEI | P *
|-----------------------|------|---------|------
| Change from baseline to POD1 | 3.1±0.9 | 1.7±0.6 | 0.044
| Usual- to high-dose lisinopril, n | 6 | 8 | 0.044
| Change from baseline to POD1 | 3.0±0.8 | 2.8±0.7 | 0.474

---

**TABLE 4. Postoperative Clinical Outcome**

| Dysrhythmia, n (%) | ACEI (n=19) | No ACEI (n=12) | P *
|-------------------|-------------|---------------|------
| Ventricular | 3 (15.8) | 0 | 0.265
| AV block | 1 (5.3) | 2 (16.7) | 0.543

---

**Study Limitations**

Although patients were randomized to preoperative ACE inhibition, the present study was neither blinded nor placebo-controlled. In addition, the dose and class of ACEI were not standardized. Nevertheless, all but 3 patients were taking lisinopril. Moreover, previously published reports that enalapril,33 quinapril,8 and ramipril9 (carboxyl-containing ACEIs like lisinopril), fosinopril11 (a phosphinyl-containing ACEI), and captopril14 (a sulphydryl-containing ACEI) all decrease PAI-1 concentrations suggest that the effect of ACE inhibition on PAI-1 is a class effect. The finding that lisinopril decreased postoperative PAI-1 in the present study confirms
a study by Shinosaki et al., who reported that lisinopril suppressed PAI-1 expression in an animal model of mesangio proliferative nephritis but conflicts with a study by Zehetgruber et al., who reported no effect of lisinopril on fibrinolysis. Data from the present study suggest that the effect of lisinopril on PAI-1 was dose-dependent. When patients who received lisinopril were analyzed by dose level, postoperative PAI-1 antigen concentrations were suppressed in patients taking 20 to 40 mg/d lisinopril but not in patients taking ≤10 mg/d. This finding is in agreement with the findings of Pahor et al., who demonstrated that fosinopril reduces PAI-1 in a dose-dependent fashion.

In conclusion, this study demonstrates that ACE inhibition attenuates the increase in PAI-1 in a dose-dependent fashion in patients undergoing CABG requiring CPB, suggesting a role for ACE inhibition in reducing the risk for acute graft thrombosis. Future clinical trials are needed to determine the impact of ACEI on early graft occlusion.

Acknowledgments

This research was funded by National Institutes of Health grants HL-60906, HL-65193, and HL-04445. Dr Pretorius is the recipient of a VA research career development award. We would like to thank Rhoda Jones, BS (Vanderbilt University), for her technical assistance.

References

Angiotensin-Converting Enzyme Inhibition Alters the Fibrinolytic Response to Cardiopulmonary Bypass
Mias Pretorius, Laine J. Murphey, Julie A. McFarlane, Douglas E. Vaughan and Nancy J. Brown

_Circulation_. 2003;108:3079-3083; originally published online December 1, 2003;
doi: 10.1161/01.CIR.0000105765.54573.60
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/25/3079

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/