The fibrinolytic response to cardiopulmonary bypass (CPB) is biphasic. An initial hyperfibrinolytic phase is characterized by a rapid increase in plasma tissue-type plasminogen activator (t-PA) concentrations and bleeding. This is followed by a postoperative hypofibrinolytic phase associated with increased plasminogen activator inhibitor-1 (PAI-1) expression and decreased circulating t-PA. Disruption of fibrinolytic homeostasis results in hemorrhage during excessive fibrinolysis and thrombosis and inflammation during inappropriate fibrinolytic inhibition. Simultaneous with the fibrinolytic response, CPB induces a systemic inflammatory response characterized by interleukin (IL) production. Because increased PAI-1 and IL-6 concentrations are associated with an increased risk of postoperative atrial fibrillation, infection, and acute kidney injury, drugs that alter the acute-phase response to CPB might decrease postoperative morbidity.

Inhibition of the renin–angiotensin system by angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor blockers (ARBs) has been shown to decrease inflammation in patients with hypertension and rheumatoid arthritis. We previously demonstrated that continued ACE inhibition increases intraoperative bradykinin and tissue-type plasminogen activator (t-PA) concentrations as compared to ARB. Both ACE inhibition and ARB decreased the need for plasma transfusion relative to placebo, but only ACE inhibition decreased the duration of hospital stay. Neither ACE inhibition nor ARB significantly affected concentrations of plasminogen activator inhibitor-1 (PAI-1), interleukin (IL)-6, IL-8, or IL-10. ACE inhibition enhanced intraoperative fibrinolysis without increasing the likelihood of red-cell transfusion. By contrast, neither ACE inhibition nor ARB affected the inflammatory response. ACE inhibitors and ARBs may be safely continued until the day of surgery.
inflammatory response, whereas other studies found no effect or an enhancement of the inflammatory response. A study of perioperative ARB therapy failed to show a significant effect of ARB on IL-6 concentrations.

This study tested the hypothesis that perioperative ACE inhibition enhances fibrinolysis and inflammation to greater extent than ARB in patients undergoing CPB.

RESULTS

Patient demographics

A total of 111 patients consented to participate in the study (Figure 1). Of these, 2 patients did not meet inclusion/exclusion criteria, 17 subjects withdrew prior to randomization, and surgery was canceled in 5 subjects. The other 87 subjects were randomly assigned to one of three groups, but 13 were subsequently excluded for the following reasons: 4 subjects changed their minds about participation and withdrew after randomization but prior to taking study medication; 3 subjects did not receive the study drug in time; 1 subject's surgery date was changed; 1 subject's surgery was canceled; 2 subjects experienced low blood pressure and weakness and stopped taking study drug; 1 subject's creatinine level rose above 1.6 mg/dl prior to surgery; and 1 subject underwent surgery not involving CPB. Ultimately, 74 subjects completed the study protocol and were included in the final analysis.

There were no significant differences among the three treatment groups in baseline subject characteristics (Table 1). Preoperative ACE activity (measured prior to CPB) was significantly lower in the ramipril group (8.1 ± 1.0 U/l) relative to the placebo group (32.4 ± 5.1 U/l, P < 0.001 vs. ramipril) and also relative to the candesartan group (29.0 ± 2.9 U/l; P < 0.001 vs. ramipril), indicating effective inhibition of ACE activity by ramipril. Five mg/day ramipril inhibited ACE activity by 75%. This is consistent with the findings of a previous study in which ramipril 2.5, 5, or 10 mg significantly decreased ACE activity relative to baseline by an average of 71% among all dose groups.

Intraoperative subject characteristics and postoperative outcomes

There were no significant differences among study groups with respect to type of surgery, CPB time, cross-clamp time, use of aortic cross-clamp, use of hemodilution during CPB, or use of steroids in the CPB pump prime (Table 2). In addition, isotropic support and use of vasopressors were similar among treatment groups. Blood loss, as measured by chest tube output and need for surgical re-exploration, total fluids in and out, postoperative atrial fibrillation, acute kidney injury, and mortality were not significantly different among treatment groups (Table 3). The blood-product transfusion exposure was similar among treatment groups, except that fresh frozen plasma was more frequently transfused in the placebo group. The duration of hospital stay was significantly shorter in subjects randomized to receive ACE inhibitor therapy.

Effect of CPB on the fibrinolytic and inflammatory response

CPB was associated with a significant increase in t-PA antigen (from 13.5 ± 0.9 ng/ml preoperatively to 35.6 ± 1.9 ng/ml post-CPB, P < 0.001) and PAI-1 antigen (from 16.4 ± 1.3 ng/ml preoperatively to 43.1 ± 3.2 ng/ml post-CPB, P < 0.001). The PAI-1:t-PA molar ratio decreased significantly (from 1.9 ± 0.1 preoperatively to 1.3 ± 0.1 at 60 min of CPB, P < 0.001), reflective of the initial profibrinolytic phase of CPB. CPB was also associated with increased expression of the proinflammatory markers IL-6 (from 7.3 ± 1.2 pg/ml preoperatively to a peak concentration of 206.0 ± 38.1 pg/ml on postoperative day 1 (POD 1), P < 0.001), IL-8 (from 14.0 ± 1.6 pg/ml post-CPB to a peak concentration of 73.9 ± 10.2 pg/ml post-CPB, P < 0.001), and the anti-inflammatory marker IL-10 (from 4.7 ± 0.4 pg/ml preoperatively to a peak concentration of 662.2 ± 111.2 pg/ml post-CPB, P < 0.001).

Figure 1  Study enrollment. BP, blood pressure.
Effect of study drug on the fibrinolytic and inflammatory response

Preoperative levels of t-PA antigen, PAI-1 antigen, and the PAI-1:t-PA molar ratio were not significantly different among treatment groups (all P values >0.44). Although the study drug did not significantly affect t-PA antigen concentrations over time (P = 0.28 for the effect of study drug over time, Figure 2a), we observed a trend for the interaction between study drug and t-PA over time. Because of this interaction trend, and the fact that the fibrinolytic response after CPB surgery is characterized by an initial hyperfibrinolytic intraoperative phase and a subsequent hypofibrinolytic postoperative phase, we investigated the effect of study drug on intraoperative and postoperative t-PA antigen concentrations separately, using the Wilcoxon rank-sum test. Ramipril significantly increased post-CPB t-PA antigen concentrations and decreased post-CPB PAI-1:t-PA antigen molar ratios compared to candesartan. Study drug did not affect PAI-1 antigen concentrations (P = 0.84 for effect of study drug, Figure 2b). Preoperative use, Hemoconcentrator use, n (%)

| Characteristics | Placebo (n = 28) | Ramipril (n = 24) | Candesartan (n = 22) | P value |
|-----------------|-----------------|-------------------|----------------------|---------|
| Hypertension    | 21 (75.0)       | 17 (70.8)         | 18 (81.8)            | 0.68    |
| Current atrial fibrillation | 11 (39.3) | 8 (33.3) | 9 (40.9) | 0.85 |
| Diabetes        | 9 (32.1)        | 4 (16.7)          | 9 (40.9)             | 0.19    |
| Past smoking    | 11 (39.3)       | 10 (41.7)         | 9 (40.9)             | 0.98    |
| COPD            | 3 (10.7)        | 4 (16.7)          | 3 (13.6)             | 0.82    |

Medications prior to randomization, N (%)

| Characteristics | Placebo (n = 28) | Ramipril (n = 24) | Candesartan (n = 22) | P value |
|-----------------|-----------------|-------------------|----------------------|---------|
| ACEi            | 8 (28.6)        | 11 (45.8)         | 8 (36.4)             | 0.44    |
| ARB             | 6 (21.4)        | 2 (8.3)           | 3 (13.6)             | 0.41    |
| β-Blocker       | 19 (67.9)       | 13 (54.2)         | 9 (40.9)             | 0.16    |
| Statin          | 17 (60.7)       | 14 (58.3)         | 9 (40.9)             | 0.33    |
| Diuretic        | 17 (60.7)       | 10 (41.7)         | 14 (63.6)            | 0.25    |
| Calcium-channel blocker | 5 (19.2) | 7 (29.2) | 7 (31.8) | 0.57 |

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; SBP, systolic blood pressure.

**Clinical correlates**

Post-bypass PAI-1 antigen levels (all subjects) correlated with duration of CPB (r² = 0.31, P < 0.001; Figure 4a) and were higher in subjects who underwent aorta cross-clamping than in those who did not (49.5 ± 4.9 ng/ml vs. 34.3 ± 3.0 ng/ml, P = 0.03). Mixed-effects models that included type of surgery (valve, coronary artery bypass graft, or combined surgery) as a covariate indicated an association between surgery type and post-bypass PAI-1 antigen levels (P = 0.002), but no association between surgery type and t-PA antigen or PAI-1:t-PA molar ratio. Including type of surgery in the model did not impact the effect of study drug on these biomarkers.

As was observed for PAI-1 antigen, post-bypass IL-6 concentrations (all subjects) correlated with duration of CPB (r² = 0.69, P = 0.001, Figure 3a–c). IL-6, IL-8, and IL-10 concentrations were similar among treatment groups (all P values >0.57). Study drug had no effect on IL-6, IL-8, or IL-10 concentrations over time (P = 0.69, P = 0.97, and P = 0.46, respectively, for effect of study drug over time, Figure 3a–c).
Bradykinin concentrations increased 2.7-fold in the entire cohort, from 51.5 ± 4.7 fmol/ml before surgery to 139.6 ± 23.7 fmol/ml post-CPB (P < 0.001). Post-CPB bradykinin concentrations were higher in the ramipril group as compared with the candesartan group (175.4 ± 61.0 vs. 113.3 ± 28.7 fmol/ml, P = 0.03), and bradykinin concentrations during CPB correlated with t-PA antigen concentrations (r² = 0.23, P < 0.001). Peak bradykinin concentrations did not correlate with post-bypass IL-6, IL-8, or IL-10 concentrations (all P values >0.43).

**DISCUSSION**

This prospective, placebo-controlled, randomized clinical trial revealed four findings about the role of the renin-angiotensin system during CPB in adult patients without severe chronic kidney disease or severe systolic heart failure: (i) ACE inhibition enhances intraoperative fibrinolysis (increases t-PA antigen and decreases PAI-1:t-PA molar ratio) without increasing blood loss or transfusion exposure risk, (ii) neither ACE inhibition nor ARB affect PAI-1 concentrations, (iii) perioperative ACE inhibition or ARB do not significantly affect CPB-induced IL response, and (iv) although it is common practice to stop ACE inhibitors and ARBs several days before surgery involving CPB, this study provides new data suggesting that the continuation of these drugs is safe.

**Fibrinolysis**

Most studies in patients in a nonsurgical setting suggest that chronic ACE inhibition decreases PAI-1 concentrations whereas ARB has no effect. In contrast to the findings of our previous studies in patients undergoing surgery, the present study showed that perioperative ACE inhibition did not attenuate the increase in postoperative PAI-1 concentrations. This finding may be explained in terms of a difference in study methodologies. The current clinical trial consisted mostly of patients undergoing heart valve surgery randomized to a single, fixed-dose ACE inhibitor, whereas in our previous studies the patients had been on chronic diverse ACE inhibitor therapies at different doses before they were randomized to either continue or discontinue their current ACE inhibitor therapy. Although the dosage of the ACE inhibitor could influence the PAI-1 response, the HEART study investigators reported that both low-dose and full-dose ramipril decrease PAI-1 to similar extents. The dose of ramipril in this study, although not the maximum one, was sufficient to suppress ACE activity as compared with the ARB and placebo groups. We cannot exclude the possibility that our results might have been different if we had studied only patients on chronic ACE inhibitor therapy. Taken together with the results of prior studies, the current findings suggest that short-term ACE inhibition does not affect the PAI-1 response, whereas withdrawal of chronic ACE inhibition may lead to an increase in PAI-1 response after CPB. The effect of ARBs on the fibrinolytic response after CPB has not been studied. ARB treatment did not affect PAI-1 concentrations. The absence of any observed effect of ARBs on the fibrinolytic response may be attributable to non-angiotensin II type 1 receptor subtypes mediating the effect of angiotensin II on endothelial PAI-1 expression. Consistent with our previous studies, this study showed that perioperative ACE inhibition increases t-PA levels during cardiac surgery.

**Inflammatory response**

CPB was associated with a significant increase in postoperative inflammatory markers. Neither ACE inhibition nor use of ARBs significantly affected the increase in postoperative pro- or anti-inflammatory markers. In prior studies, preoperative ACE inhibition has been associated with decreased, increased, or unchanged cardiac surgery–induced inflammatory response.

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**Author Contributions**

All authors contributed to the conception and design of the study, acquisition and interpretation of the data, and drafting of the manuscript.

**Conflicts of Interest**

No conflicts of interest are declared.

**Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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**Table 3** Postoperative characteristics

| Morbidity, n (%) | Placebo (n = 28) | Ramipril (n = 24) | Candesartan (n = 22) | P value |
|-----------------|------------------|-------------------|----------------------|---------|
| Acute renal failure (STS criteria)a | 0 | 1 (4.2) | 1 (4.5) | 0.53 |
| Acute kidney injury (stage Ib) | 8 (28.6) | 5 (23.8) | 8 (36.4) | 0.51 |
| Re-exploration | 1 (3.6) | 2 (8.3) | 1 (4.5) | 0.73 |
| Stroke | 2 (7.1) | 1 (4.2) | 1 (4.5) | 0.87 |
| New-onset atrial fibrillation | 5 (17.9) | 4 (16.7) | 6 (27.3) | 0.62 |
| Pacemaker placement | 6 (21.4) | 1 (4.2) | 2 (9.1) | 0.14 |
| Length of hospital stay (days) | 7.7 ± 0.5 | 6.3 ± 0.6* | 8.1 ± 1.0 | 0.04 |
| In-hospital mortality, n (%) | 0 | 0 | 1 (4.5) | 0.30 |

*P < 0.05 vs. placebo.

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**References**

1. **Fibrinolysis**

   Most studies in patients in a nonsurgical setting suggest that chronic ACE inhibition decreases PAI-1 concentrations whereas ARB has no effect. In contrast to the findings of our previous studies in patients undergoing surgery, the present study showed that perioperative ACE inhibition did not attenuate the increase in postoperative PAI-1 concentrations. This finding may be explained in terms of a difference in study methodologies. The current clinical trial consisted mostly of patients undergoing heart valve surgery randomized to a single, fixed-dose ACE inhibitor, whereas in our previous studies the patients had been on chronic diverse ACE inhibitor therapies at different doses before they were randomized to either continue or discontinue their current ACE inhibitor therapy. Although the dosage of the ACE inhibitor could influence the PAI-1 response, the HEART study investigators reported that both low-dose and full-dose ramipril decrease PAI-1 to similar extents. The dose of ramipril in this study, although not the maximum one, was sufficient to suppress ACE activity as compared with the ARB and placebo groups. We cannot exclude the possibility that our results might have been different if we had studied only patients on chronic ACE inhibitor therapy. Taken together with the results of prior studies, the current findings suggest that short-term ACE inhibition does not affect the PAI-1 response, whereas withdrawal of chronic ACE inhibition may lead to an increase in PAI-1 response after CPB. The effect of ARBs on the fibrinolytic response after CPB has not been studied. ARB treatment did not affect PAI-1 concentrations. The absence of any observed effect of ARBs on the fibrinolytic response may be attributable to non-angiotensin II type 1 receptor subtypes mediating the effect of angiotensin II on endothelial PAI-1 expression. Consistent with our previous studies, this study showed that perioperative ACE inhibition increases t-PA levels during cardiac surgery.

2. **Inflammatory response**

   CPB was associated with a significant increase in postoperative inflammatory markers. Neither ACE inhibition nor use of ARBs significantly affected the increase in postoperative pro- or anti-inflammatory markers. In prior studies, preoperative ACE inhibition has been associated with decreased, increased, or unchanged cardiac surgery–induced inflammatory response.
another study, ARBs tended to decrease IL-6 concentrations after CPB surgery. It is difficult to compare our findings with those of these other studies because of differences in study methodology. For example, the study by Radaelli et al. consisted predominantly of male patients undergoing coronary artery bypass graft surgery; it demonstrated inflammation attenuation only with high-dose ACE inhibitor plus high-dose statin therapy, and this effect was no longer present at 6 h after cross-clamp removal. Our study population, on the other hand, underwent more diverse surgical procedures, and statin use was not randomized. Despite our use of a lower dose of ramipril, ACE activity was significantly suppressed even at this dose. The majority of the postoperative IL-6 variability observed in our study was secondary to duration of the CPB procedure, aortic cross-clamping, type of surgery,

**Figure 2** Effects of study drug on markers of fibrinolysis. (a) Tissue-type plasminogen activator (t-PA) antigen concentrations. (b) Plasminogen activator inhibitor-1 (PAI-1) antigen concentrations. (c) PAI-1:t-PA molar ratios. 60 min, 60 min of cardiopulmonary bypass surgery; POD, postoperative day; Post, post-CPB; Preop, preoperative.
and administration of steroids. Even including these variables in the mixed-effects model did not reveal any effect of renin-angiotensin blockade on the inflammatory response.

Bradykinin concentrations were measured to investigate the contributions of bradykinin to the inflammatory response, given that bradykinin has been shown to increase circulating IL-6,13,25 and both ACE inhibition, and, to a lesser extent, ARB increase bradykinin concentrations by decreasing bradykinin metabolism.14,26 As expected, ACE inhibition increased bradykinin concentrations,27 and, although increased levels of bradykinin correlated with increased t-PA concentrations in our study, there were no significant associations between bradykinin and other inflammatory marker concentrations. Ongoing studies of the effects of a perioperative bradykinin receptor antagonist (NCT00223704) will help elucidate the role of bradykinin in postoperative inflammation.

**Clinical outcomes**
In the current study, we made the additional observation that increased markers of intraoperative fibrinolysis are not necessarily associated with increased postoperative blood loss or increased likelihood of requiring blood-product transfusion. In fact, ACE inhibition was associated with a lower requirement for fresh frozen plasma as compared with placebo. Between-group differences in other predictors of coagulopathy, hemorrhage, and transfusion, such as surgical hemostasis, platelet dysfunction,
but may have led to the little-observed effect of renin–angiotensin system blockade on clinical fibrinolytic and inflammatory markers. We cannot exclude the possibility that our results might have been different if we had studied a homogeneous surgery population. However, controlling for the type of surgery did not change the effect of study drug on clinical fibrinolytic and inflammatory markers. On the other hand, our diverse study population allows for generalizability of our study results to diverse cardiac surgical populations.

In conclusion, this study demonstrates that perioperative ACE inhibition increases intraoperative t-PA and bradykinin concentrations but does not increase blood loss or transfusion exposure risk, that perioperative ARBs do not affect plasma markers or clinical indicators of fibrinolysis, and that neither ACE inhibition nor ARBs alter the inflammatory response to CPB. The modest effect of ACE inhibition on the fibrinolytic balance (with no increase in detrimental effects such as blood loss or requirement for blood product transfusion) and the absence of a significant effect on the inflammatory response suggests that inhibition of the renin–angiotensin system plays only a minor role in the postoperative fibrinolytic and inflammatory response and that ACE inhibitors and ARBs may be safely continued until the day of surgery. Additional prospective trials are needed to assess the impact of preoperative ACE inhibition and ARBs on clinical outcomes.

METHODS
Adult patients scheduled for cardiac surgery involving CPB were eligible for the study. Exclusion criteria included left ventricular ejection fraction <30%, serum potassium >5.0 mEq/l, serum creatinine >1.6 mg/dl, and inability to discontinue current ACE inhibitor or ARB therapy.

One week to 5 days prior to surgery, patients were randomized to treatment with placebo, ramipril (2.5 mg on the first 3 days followed by 5 mg/day, with the dose reduced to 2.5 mg/day on the first POD only), or candesartan (16 mg/day). The 5-mg daily dose of ramipril was chosen to achieve significant inhibition of ACE activity without compromising patient safety (a larger dose of ramipril could increase perioperative hypotension and the withholding of study drug protocol violations). Randomization was stratified on the basis of prior ACE inhibitor and ARB use. Preexisting ACE inhibitor and ARB therapies were stopped at randomization. All other preoperative medications were continued until the day of surgery. Safety criteria for stopping the study medication were hypotension (defined as systolic blood pressure <90 mm Hg or prolonged need for vasopressors), serum potassium >5.5 mEq/l (if confirmed by a repeat measurement), and acute renal failure, defined using the criteria of the Society of Thoracic Surgery, which defines postcardiac surgery acute renal failure as serum creatinine concentration ≥2.0 mg/dl and at least twice the baseline value. The study (ClinicalTrials.gov identifier NCT00607672) was approved by the Vanderbilt University Human Research Protection Program and the Tennessee Valley Healthcare System Institutional Review Board and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Primary and secondary end points. The primary end points were (i) the effect of the study drug on the fibrinolytic response, as quantified by measurement of plasma t-PA and PAI-1 antigen concentrations, and (ii) the effects of the study drug on the inflammatory response, as quantified by measurement of plasma IL-6, IL-8, and IL-10 concentrations. Because ACE inhibition has been shown to impact fibrinolysis, kidney injury, and atrial fibrillation, we also assessed the following secondary end points: postoperative blood loss, transfusion requirements, re-exploration for bleeding, use of inotropics and vasopressors, new-onset atrial fibrillation, and changes in serum creatinine.

Limitations
Contrary to previous studies that focused on coronary artery bypass graft–only surgical populations, we included a diverse study population that reflects standard cardiac practice but may have led to the little-observed effect of renin–angiotensin and hemodilution, may explain this finding. ACE inhibition was not associated with any safety concerns; on the contrary, it was associated with a decreased duration of hospital stay, suggesting that there are additional benefits to perioperative ACE inhibition. Prior retrospective studies had demonstrated either no difference or an increase in adverse clinical outcomes associated with preoperative ACE inhibition. Our study was not powered to assess the impact of ACE inhibition on clinical outcomes, and therefore clinical trials are needed to determine whether preoperative ACE inhibition impacts clinical outcomes.
Acute kidney injury was defined in accordance with the Acute Kidney Injury Network criteria, specifically, any increase of 50% or 0.3 mg/dl (26.5 μmol/l) in serum creatinine concentration within 72 h of surgery. The network’s urine-output criteria for acute kidney injury were not used because of confounding by intravascular hypovolemia and use of diuretics, both of which are common among cardiac surgery patients.

Standardized patient treatment. Anesthesia management and CPB were conducted according to institutional protocols. Induction of anesthesia was achieved with either etomidate or propofol and maintained with isoflurane, fentanyl, air, and oxygen. Muscle relaxation was achieved and maintained with pancuronium or rocuronium. Hemodynamics were invasively monitored with an arterial line (Arrow International, Reading, PA) and a pulmonary artery catheter (Edwards Lifesciences, Irvine, CA). CPB was achieved with a roller pump (Medtronic, Minneapolis, MN), a heparin-coated circuit (Carmeda), and a Trillium hollow-fiber oxygenator (Medtronic). Heparin was used for anticoagulation during CPB, at an initial dose of 300 U/kg supplemented with additional heparin to achieve and maintain an activated clotting time >400 s. All patients received t-aminocaproic acid (antifibrinolytic drug) as a bolus of 100 mg/kg over a period of 30 min prior to CPB, followed by 25 mg/kg/h throughout surgery. Temperature management included cooling to 28–30 °C, temperature-uncorrected blood-gas management (alpha stat), and cold antegrade and retrograde cardioplegia techniques when an aortic cross-clamp was applied. At the conclusion of CPB, anticoagulation was reversed with 250 mg protamine, with additional 50 mg aliquots administered to achieve baseline activated clotting time. Vasopressors were used at separation from CPB if the mean arterial blood pressure was <60 mm Hg. Inotropes were used for separation from CPB in the presence of any of the following: left ventricular ejection fraction <40%, CPB surgery duration >120 min, cardiac index <2 l/min·m², or evidence of new-onset left ventricular dysfunction as shown by transthoracal echocardiography. The use of inotropes and/or vasopressors in the postoperative period was at the discretion of the intensive-care physicians. Patients were given transfusions in accordance with the following guidelines: packed red blood cells were transfused if the hematocrit level was <20% during CPB or <25% after CPB. Platelets were transfused in 5–U sets for ongoing microvascular bleeding despite a normalized activated clotting time or when CPB duration was >120 min. Fresh frozen plasma was transfused if the international normalized ratio was >1.5 or there was continued bleeding even after platelets were given. Transfusion requirements were recorded from the beginning of surgery to the time of discharge from the hospital.

Assays. Blood samples were collected to measure ACE activity, PAI-1, t-PA antigen, and inflammatory markers at five time points: (i) after induction of anesthesia and prior to surgical incision (preoperative), (ii) at 60 min into CPB, (iii) after separation from CPB and administration of protamine (post-bypass), (iv) on POD 1, and (v) on POD 2. All blood samples were drawn from the indwelling arterial line. Not all markers were assayed at each time point. Blood samples for measurement of PAI-1 and t-PA were collected in vacutainer tubes containing 0.5 ml 0.5 mol/l citrate buffer (Tcoga Ireland Ltd., Bray Co., Wicklow, Ireland). All blood samples were collected on ice and centrifuged immediately at 4 °C for 20 min. Plasma samples were stored at −80 °C until assay. The levels of PAI-1 antigen (TriniLIZE PAI-1 Antigen; Tcoga Ireland) and t-PA antigen (TriniLIZE t-PA Antigen; Tcoga Ireland) were determined using a two-site, enzyme-linked immunosorbent assay, as previously described. The PAI-1-t-PA molar ratio (an indicator of fibrinolytic balance) was determined assuming a molecular weight of 70,000 g/mol for t-PA and 50,000 g/mol for PAI-1. Blood samples for measurement of bradykinin were drawn into cold anhydrous ethanol (4:1 blood to ethanol). After 1 h at 4 °C, the mixture was centrifuged (15 min, 3,000 r.p.m., 10 °C), and the supernatant was decanted and frozen at −80 °C until assay. Bradykinin concentrations were determined using a commercially available enzyme immunoassay (Peninsula Laboratories, Division of Bachem, San Carlos, CA). A panel of human inflammatory cytokines consisting of IL-6, IL-8, and IL-10 was simultaneously measured by the Vanderbilt University Immunology Core laboratory using the Human Inflammation Cytokine Cytometric Bead array kit (BD Biosciences Pharmingen, San Diego, CA). Serum ACE activity was determined using a three-step colorimetric assay in which ACE hydrolyzes the substrate p-hydroxybenzoyl-glycyl-l-histidyl-l-leucine and subsequent reactions lead to the formation of quinoneimine dye, which was measured using spectrophotometry (Fujirebio America, Fairfield, NJ).

Statistical analysis. Data are presented as mean values ± s.e.m. unless otherwise indicated. Based on preliminary data, we powered the study to detect a 16-ng/ml difference in POD 1 PAI-1 between the placebo group and either the ARB group or the ACE inhibitor group, assuming a standard deviation of 20 ng/ml. Twenty-six subjects per group provides 80% power with a 0.05 two-sided significance level. Discrete variables were compared among treatment groups using the χ²-test or Fisher’s exact test depending on the number of events for three-group or pairwise comparisons. Continuous data were compared among treatment groups using one-way analysis of variance or the Kruskal–Wallis test, depending on the normality of the data. Correlations were evaluated using the Spearman’s rank or Pearson correlation coefficient, depending on the normality of the data. Longitudinal measures of PAI-1, t-PA, PAI-1-t-PA ratio, and ILs were analyzed using mixed-effects models with drug treatments (placebo, ramipril, or candesartan) and time since randomization as fixed effects. We included a random subject effect and a first-order autoregressive process to adjust for any errors in the mixed-effects model. In separate mixed-effects models, CPB duration, type of surgery, use of aortic cross-clamp, and/or use of steroids in the CPB pump prime were included to adjust for their potential effects on the inflammatory response. Predictors of post-bypass IL-6 concentrations were additionally assessed using linear regression. CPB duration, type of surgery, use of aortic cross-clamp, and use of steroids in the pump prime were included in this model as independent variables. A two-tailed P value <0.05 was considered statistically significant. Statistical analyses were performed using the statistical package IBM SPSS for Windows (version 19.0; IBM, New York, NY) and SAS for Windows (Version 9; SAS, Cary, NC).

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AUTHOR CONTRIBUTIONS
F.T.B. wrote the manuscript. J.M.B. wrote the manuscript. C.Y. wrote the manuscript, designed research, and analyzed data. P.W. performed research. M.R.P. wrote the manuscript. J.G.B. wrote the manuscript. N.J.B. wrote manuscript and designed research. M.P. wrote the manuscript, designed research, and analyzed data.

CONFLICT OF INTEREST
The authors declared no conflict of interest.

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