Comparative Effects of Angiotensin-Converting Enzyme Inhibition and Angiotensin-Receptor Blockade on Inflammation during Hemodialysis

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ABSTRACT

Biomarkers of oxidative stress and inflammation predict cardiovascular events in maintenance hemodialysis patients. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs) reduce cardiovascular mortality in the general population, but their benefit in maintenance hemodialysis patients is not fully explored. To test whether ACE inhibitors and ARBs differentially affect markers of oxidative stress, inflammation, and fibrinolysis during hemodialysis, we conducted a randomized, double-blind, placebo-controlled 3×3 crossover study. We randomly assigned 15 participants undergoing hemodialysis to placebo, ramipril (5 mg/d), and valsartan (160 mg/d) for 7 days, with a washout period of 3 weeks in between the treatments. On the morning of the seventh day of drug treatment, participants underwent serial blood sampling during hemodialysis. Neither ramipril nor valsartan affected BP during hemodialysis. Ramipril increased IL-1β concentrations (P=0.02) and decreased IL-10 concentrations (P=0.04) compared with placebo. Valsartan and ramipril both lowered IL-6 levels during dialysis (P<0.01 for each compared with placebo). Valsartan increased F2-isoprostane levels, and ramipril suggested a similar trend (P=0.09). Valsartan and ramipril both lowered D-dimer levels (P<0.01 for both), whereas only ramipril seemed to prevent a rise in vWf levels (P=0.04). In summary, during hemodialysis, valsartan induces a greater anti-inflammatory effect compared with ramipril, although ramipril seems to prevent dialysis-induced endothelial dysfunction as measured by levels of vWF. A prospective clinical trial is necessary to determine whether ACE inhibitors and ARBs also differ with respect to their effects on cardiovascular mortality in this population.


In ESRD patients, cardiovascular death accounts for >50% of overall mortality and the risk of cardiovascular death is 30 times higher than in the general population.1,2 Moreover, patients undergoing maintenance hemodialysis (MHD) have a 5-year survival rate of 10% after acute myocardial infarction.3 Traditional risk factors, as used in Framingham risk scoring, only explain half of the excessive cardiovascular mortality observed in ESRD patients,4 and it is thought that other factors contribute to the high cardiovascular mortality among ESRD patients.

Increased oxidative stress and inflammation may contribute to accelerated atherosclerosis and cardiovascular events in patients with MHD.5,6 Markers of oxidative stress, such as F2-isoprostanes, are increased in MHD patients.7,8 Inflammatory markers, particularly IL-1, IL-6, and TNFα, are also elevated in MHD patients and are associated with
increased risk of mortality. Both underlying uremia itself and recurrent hemodialysis can contribute to inflammation. Hemodialysis induces an acute increase in white blood cellular transcription and circulating concentrations of inflammatory markers. Inflammatory cytokines, in turn, increase the expression of prothrombotic factors, such as plasminogen activator inhibitor-1 (PAI-1), the major inhibitor of fibrinolysis by inactivating the tissue plasminogen activator (tPA). Increased PAI-1 antigen levels and activity in MHD patients correlate with increased risk of coronary artery disease.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs) reduce cardiovascular mortality in the general population, but a single prospective clinical trial of the ACE inhibitor fosinopril in MHD patients showed no protective effect on cardiovascular events. This trial may have been underpowered to detect an effect of ACE inhibition. Another trial in MHD patients with chronic heart failure showed that adding the ARB telmisartan to their medication regimen decreased all-cause mortality, cardiovascular mortality, and duration of hospitalization.

In a recent retrospective observational study in MHD patients, initiation of an ACE inhibitor plus other antihypertensive medication was associated with increased mortality compared with initiation of an ARB and other antihypertensive medication. ACE inhibitors and ARBs both reduce angiotensin II activity but they differ in their effects on bradykinin. ACE inhibitors, but not ARBs, increase bradykinin bioavailability by reducing its degradation. Bradykinin increases tPA and inhibits platelet aggregation, but also increases oxidative stress and inflammation. Hemodialysis also stimulates activation of the kallikrein-kinin system and the production of bradykinin. Endogenous bradykinin contributes to increases in PAI-1 and monocyte chemoattractant protein-1 after hemodialysis. Thus, any intervention that increases bradykinin, such as ACE inhibitors, may increase the inflammatory response to hemodialysis in MHD patients.

In this study, we tested the hypothesis that short-term administration of ACE inhibitors and ARBs would differ in their effects on oxidative stress, inflammation, and fibrinolysis in response to hemodialysis in MHD patients. We conducted a randomized, double-blind, placebo-controlled 3 × 3 crossover study comparing the effect of 1-week treatment with the ACE inhibitor ramipril, the ARB valsartan, and placebo on the responses to hemodialysis.

**RESULTS**

**Patient Characteristics**

Table 1 shows baseline patient characteristics. The causes of ESRD were hypertension (9 of the 15 patients), diabetes mellitus (4 patients), nephropathy induced by nonsteroidal anti-inflammatory drugs (1 patient), and unknown (1 patient).

**Effects of Hemodialysis and Treatment on Hemodynamics and Renin-Angiotensin System Parameters**

Baseline mean arterial BP (MABP) was comparable among the treatment arms and MABP decreased significantly and similarly during hemodialysis (from 97.9 ± 3.65 at the beginning to 88.16 ± 3.43 mmHg at the end of dialysis, Figure 1) among all three treatments. Heart rate increased significantly and similarly during dialysis but there was no effect of treatment (Figure 1). ACE activity was significantly lower during ramipril treatment compared with during placebo or valsartan treatment (Figure 2), and did not change during hemodialysis.
Valsartan increased ACE activity when compared with placebo at the end of hemodialysis (Figure 2). Plasma renin activity (PRA) was significantly higher during treatment with ramipril or valsartan compared with placebo. PRA increased during dialysis with ramipril and valsartan treatments but not placebo ($P=0.04$ for time $\times$ drug interaction, Figure 2). We detected no evidence for a carry-over effect on ACE activity or PRA. Bradykinin concentrations were increased during ramipril treatment compared with during placebo or valsartan (Figure 2).

**Effect of Hemodialysis and Treatment on Inflammation and Oxidative Stress**

IL-1$\beta$ levels were significantly higher during ramipril treatment compared with placebo (Figure 3). Serum IL-6 and IL-8 concentrations were lower during valsartan and ramipril treatment compared with placebo, and did not change with dialysis (Figure 3 and Table 2). IL-10, an anti-inflammatory cytokine, increased during dialysis (Figure 3); however, IL-10 concentrations initially decreased and remained significantly lower during ramipril treatment compared with placebo (Figure 3). Plasma F$_2$-isoprostanes concentrations were significantly higher during valsartan treatment compared with placebo and there was a trend toward higher levels during ramipril treatment compared with placebo (Figure 3).

**Effect of Hemodialysis and Treatment on Markers of Coagulation, Fibrinolysis, and Endothelial Injury**

Concentrations of tPA increased during dialysis and were similar during all three treatments (Table 3). PAI-1 concentrations were also similar during all three treatments. D-dimers, a product of fibrinolysis and marker of thrombosis, were significantly lower during valsartan and ramipril treatment compared with placebo (Figure 4). Soluble CD40 ligand (sCD40L) is a platelet-derived cytokine important for thrombi stabilization. SCD40L concentrations increased throughout dialysis during all treatments (from $91.4\pm8.5$ to $118.9\pm11.2$ ng/ml, beginning to end of dialysis, respectively; Figure 4) and there was no difference among the treatment arms. In contrast, plasma vWF, a marker of endothelial injury, increased during dialysis, and only ramipril treatment significantly attenuated this effect (Figure 4).

**DISCUSSION**

This randomized study tested the hypothesis that ACE inhibitors and ARBs differ in their effects on oxidative stress, inflammation, and fibrinolysis in MHD patients. The major findings are that although short-term treatment with an ACE inhibitor or ARB decreased IL-6 and IL-8, ramipril also exerted a
proinflammatory effect during hemodialysis, as measured by an increase in IL-1β and a decrease in the anti-inflammatory cytokine IL-10. Both ramipril and valsartan decreased the marker of thrombosis, D-dimers, but ramipril also prevented a rise in vWF, a marker of endothelial damage, during hemodialysis. These effects are not attributable to an antihypertensive effect of ramipril or valsartan, because neither drug affected BP in the MHD studied participants.

Increased oxidative stress and inflammation are commonly observed in MHD patients. The etiology of inflammation is complex and includes underlying uremia and the continuous contact between blood and dialyzer/dialysate. Indeed, the hemodialysis procedure results in increased leukocyte transcript levels of many proinflammatory cytokines13 and circulating cytokines increase and remain high at least 2 hours after the end of dialysis.12 In our study, valsartan and ramipril reduced the levels of IL-6 and IL-8 during hemodialysis. Angiotensin II stimulates IL-6 production and release31–33; therefore, ramipril and valsartan have likely reduced IL-6 by preventing the formation or action of angiotensin II. The finding that ramipril treatment increased levels of IL-1β and decreased IL-10, an anti-inflammatory cytokine, however, suggests that ramipril had an additional proinflammatory effect. One potential explanation for the differential effects on the inflammatory response between ACE inhibitors and ARBs may relate to their effect on bradykinin metabolism. ACE inhibition enhances bradykinin effects by decreasing its breakdown, whereas ARBs do not. In addition, hemodialysis increases bradykinin levels, albeit to lesser extent with polysulfone membranes than polycrylonitrile membranes. Supporting a proinflammatory role of bradykinin is the observation that bradykinin B2-receptor blockade increases circulating IL-10 in an animal model of ischemia/reperfusion.34 Furthermore, in MHD patients, bradykinin B2-receptor blockage raises IL-10 levels during hemodialysis.29 In this study, we found that bradykinin concentrations were increased during ramipril
treatment compared with during placebo or valsartan. Taken together, these data suggest that increased endogenous bradykinin accounts for lower IL-10 levels during ramipril treatment. The relation between bradykinin and IL-1β is complex. Bradykinin increases the release and gene expression of IL-1β35–37 and may enhance the effects of IL-1β.38 On the other hand, IL-1β induces the expression of bradykinin receptors.39

Inflammatory cytokines predict cardiovascular endpoints in ESRD patients. For example, IL-6 predicts mortality and correlates with the severity of carotid atherosclerosis.40,41 On the other hand, decreased levels of IL-10 have been described in MHD patients with carotid atherosclerotic plaques.42 Furthermore, a genotype associated with lower IL-10 production predicts cardiovascular death in MHD patients.43 These data support the hypothesis that ARBs may be preferable to ACE inhibitors in limiting cardiovascular events in MHD patients. Indeed, a recent study in MHD patients with chronic heart failure showed that the addition of telmisartan to standard therapies significantly reduces all-cause mortality, cardiovascular death, and heart failure hospital stays.19

Contrary to our initial hypothesis, F2-isoprostanes were increased during both ramipril and valsartan treatment. In contrast, a previous study showed that oxidative stress levels decreased in hemodialysis patients after 6-week treatment with valsartan.44 The discrepancy with our study may reside in the duration of the treatment. Although this study does not address the mechanism for the effect of valsartan and ramipril on F2-isoprostanes, we observed that increasing blood flow increases F2-isoprostanes in the human forearm vasculature.26 Further experiments may explain the relation between angiotensin II, bradykinin, and oxidative stress during hemodialysis.

Consistent with previous studies,29 we found that tPA antigen increased during dialysis. Although bradykinin stimulates the release of tPA from the endothelium, we observed no effect of ramipril on tPA concentrations during dialysis. This is in agreement with the recent results of Marney et al., who reported no effect of bradykinin B2-receptor blockade on tPA levels during hemodialysis.29 The lack of effect of bradykinin or ACE inhibitors on tPA concentrations may reflect endothelial dysfunction, commonly observed in MHD patients,45,46 because endothelial dysfunction attenuates bradykinin-stimulated tPA release.47,48

ESRD patients exhibit hypercoagulability, resulting in elevated D-dimer levels.49 Plasma D-dimers are increased in MHD, although their diagnostic value has been questioned.50 In MHD patients, D-dimers have been correlated with bleeding propensity rather than hypercoagulability51; however, D-dimers predict mortality in ESRD patients.52 The finding that either ramipril or valsartan reduced D-dimers levels in the MHD patients confirms data from clinical trials in patients without ESRD, such as the Perindopril-Thrombosis Inflammation, Endothelial Dysfunction and Neurohormonal Activation Trial (PERTINENT) and the Trial of Angiotensin-Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors.53,54 In the PERTINENT study, perindopril also improved the endothelial function, and specifically decreased vWF levels.55 This study extends this observation to demonstrate that ACE inhibition decreases acute endothelial injury during dialysis.

sCD40L is a proinflammatory and procoagulant molecule, released from platelets upon activation. sCD40L ligand concentrations are associated with an increased risk of cardiovascular events in the general population56 and are increased in patients with MHD.57 We found that sCD40L increased during dialysis and there was no difference among the treatment arms. Koh et al. also reported that 2 months of treatment with candesartan reduced the plasma levels of sCD40L in hypertensive patients.58 In contrast, studies in patients with either heart failure or atrial fibrillation failed to show a change in plasma levels of sCD40L during either ACE inhibitor or ARB treatment.59,60

This study has some limitations. It was designed to evaluate the short-term effect of valsartan and ramipril on the acute inflammatory response to hemodialysis and does not address the long-term effect of these drugs. Nevertheless, the data support a greater anti-inflammatory effect of valsartan compared with ramipril, whereas only ramipril prevented dialysis-induced endothelial dysfunction, as measured by levels of vWF. The differences between the effects of the two drugs may be attributed to bradykinin, which has proinflammatory as well as endothelial protective effects. These data suggest that results from randomized clinical trials of ACE inhibitors in MHD patients cannot be readily extrapolated to the effects of ARBs. Our results suggest that there may be specific subgroups of MHD patients that might benefit from each drug differently and emphasize the need for a long-term randomized clinical trial to compare the effects of ARBs and ACE inhibitors on cardiovascular endpoints in patients on MHD.
Study Population

This study was approved by the Vanderbilt University Institutional Review Board and performed according to the Declaration of Helsinki. Approximately 100 patients were prescreened for eligibility, 20 of whom were approached and provided written informed consent and agreed to participate in this study. Three participants were excluded on the basis of the exclusion criteria after screening (hyperkalemia, hypotension, and uncontrollable hypertension), one withdrew from the study (moved to another city), and one patient was withdrawn from the study because of stroke. (The stroke was not felt to be study related by the Data and Safety Monitoring Committee for this study.) Therefore, 15 participants completed this study. All of them underwent adequate hemodialysis (Kt/V > 1.2) three times per week for at least 6 consecutive months. Hemodialysis was performed for 4 hours using the Fresenius Optiflux 180 dialyzer (Fresenius Medical Care, Waltham, MA) with a polysulphone membrane. Dialyse quality was within the Association for the Advancement of Medical Instrumentation standards for endotoxin concentrations. In addition, dialysis was performed using Diasafe filters (Fresenius Medical Care) to ensure further purity of the dialysate. Patients were clinically stable with predialysis potassium levels < 5.5 mmol/L.

Participants with a history of functional transplant < 6 months before the study or anticipated live donor kidney transplant were excluded. Patients were excluded from the study if they had history of active connective tissue disease, acute infection within 1 month before the study, advanced liver disease, gastrointestinal dysfunction requiring parental nutrition, or active malignancy. Use of the following medications were also exclusion criteria: anti-inflammatory medication other than aspirin, 325 mg/d, immunosuppressive drugs within 1 month before the study, vitamin E, 60 IU/d, or vitamin C, 500 mg/d. Participants with a history of myocardial infarction or cerebrovascular event within 3 months before the study or an ejection fraction < 40% were excluded from this study. Other exclusion criteria were a history of ACE inhibitor–associated angioedema or cough, inability to discontinue ACE inhibitors or ARBs, pregnancy, breastfeeding, or child-bearing potential.

Study Protocol

This study used a randomized, double-blind, placebo-controlled 3×3 crossover design (Figure 5). Participants were randomized to treatment with ramipril (King Pharmaceuticals, Bristol, TN), valsartan (Novartis Pharmaceuticals, East Hanover, NJ), or placebo for 1 week. Patients receiving either ACE inhibitors or ARBs before the study discontinued that medication for 3 weeks (washout period); during this period, BP was closely monitored. If, at any time, systolic BP exceeded...
160 mmHg or diastolic BP exceeded 100 mmHg, other antihypertensive medications were maximized. If BP remained high, the protocol called for the addition of either amlodipine or clonidine; only one participant required the addition of amlodipine to control BP. After any initial washout period, participants were randomly assigned to one of the six study sequences for a 3 × 3 orthogonal Latin square design specified in Jones and Kenward. Each patient underwent three different 7-day treatment arms as follows: ramipril (initiated at 2.5 mg/d for 2 days, followed by 5 mg/d), valsartan (80 mg/d for 2 days, followed by 160 mg/d), and matching placebo. On the seventh day of treatment, patients took their medication early in the morning and reported to the Vanderbilt General Clinical Research Center for hemodialysis, blood sampling, and hemodynamic monitoring. During hemodialysis, BP was measured every 5 minutes in the arm contralateral to the vascular access using an automated device (Dinamap; Critikon, Carlsbad, CA). Blood samples were collected at the beginning of hemodialysis, 30 minutes and 1 hour after the initiation of dialysis, at the end of dialysis, and 2 hours after the completion of dialysis. After the first study day, participants entered a second washout period and completed two more cycles of treatment.

Laboratory Procedures
Blood was centrifugated immediately after collection and plasma or serum was stored at −80°C until analysis. Serum IL-1β, IL-6, IL-8, IL-10, IL-12p70, and IL-17 were measured using Luminex immunoassay technology. Monocyte chemoattractant protein-1 concentrations were measured using commercially available kits (Linco Research, St. Charles, MO). F2-isoprostanes were measured in plasma separated from EDTA-anticoagulated blood using negative ion gas chromatography mass spectroscopy as previously described. PAI-1, tPA, D-dimers, vWF, and sCD40L were measured in plasma from blood anticoagulated with 0.105 M sodium citrate. D-dimer levels were measured using a commercial kit (TintElize; Biopool, Berkeley Heights, NJ). PAI-1 and tPA antigens were determined using commercial ELISA kits (TrinILIZE; Trinity Biotech, Berkeley Heights, NJ). ELISA was also used to determine the levels of vWF (American Diagnostica Inc, Stamford, CT) and sCD40L (Quantikine; R&D Systems, Minneapolis, MN). PRA and aldosterone were determined using RIA; ACE activity was measured by kinetic analysis, as previously described. Blood for measurement of bradykinin was drawn into no-assay (Bachem; Peninsula Laboratories, San Carlos, CA).

Statistical Analyses
Data are presented as mean ± SEM. Using a coefficient of variation of 0.39 in IL-6 concentrations and a correlation of 0.7 between measurements taken in the same patient, the sample size was calculated to give 90% power to detect a 20% difference in the effect of ACE inhibitors and ARBs on IL-6 in this crossover study, on the basis of our previously reported data. We used general linear model as detailed in Chapter 5 of Jones and Kenward to test for a carry-over effect using PRA and ACE activity. Repeated-measurements ANOVA was used to analyze changes in hemodynamic parameters. Linear mixed-effect models were used to evaluate the effect of drug treatment (placebo, ramipril, and telmisartan) and time after initiation of dialysis (0, 0.5, 1, 4, and 6 hours) on biomarkers. Model selection was based in the lower Akaike’s information criterion. Sex, race, and age were included as covariates if there was evidence for an interactive effect of these variables. Bradykinin concentrations were natural log-transformed before analysis. Two-sided P < 0.05 was considered significant. Data analysis was performed using SPSS (v.17.0; SPSS Inc, Chicago, IL) and SAS for Windows (Version 9; SAS Institute, Cary, NC) software.

ACKNOWLEDGMENTS
The authors thank Ms. Delia Woods for assistance with patient recruitment, carrying out the research protocol, and data entry. We also thank Mr. Anthony DeMatteo, Zuofei Wang, and Jeff Petro for laboratory assistance.

This work was supported by NIH/NHLBI Grant R01 HL065193, NIH/NIDDK Grant K24 DK62849, a grant from Vanderbilt University’s Clinical and Translational Science Award (CTSA) program (UL1 RR024975 from the NCRR/NIH), and NIGMS/NIH Grant T32 GM007569.

DISCLOSURES
None.

REFERENCES

ACE Inhibitors, ARBs, Inflammation, and Dialysis

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