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Endothelial Function, Fibrinolysis, and Angiotensin-Converting Enzyme Inhibition

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Summary: Experimental and clinical studies with angiotensin-converting enzyme (ACE) inhibitors have suggested that these agents may reduce the risk of atherothrombotic events. Recent studies have identified the role of angiotensin II and ACE in the regulation of fibrinolysis. There is now substantial evidence that the renin-angiotensin system (RAS) plays an important role in the regulation of vascular fibrinolytic balance. This recently recognized relationship may contribute to the vasculoprotective effects of ACE inhibitors.

Key words: angiotensin-converting enzyme, fibrinolysis, plasminogen activator inhibitor type 1, tissue-type plasminogen activator

The Fibrinolytic System

The plasminogen activator, or fibrinolytic system, constitutes one of the primary endogenous mechanisms for preventing intravascular thrombosis, which is implicated importantly in the pathogenesis of myocardial infarction (MI) and other acute coronary syndromes. Fibrinolysis depends on a balance between plasminogen activators [urokinase and tissue-type plasminogen activator (TPA)] and plasminogen activator inhibitor type 1 (PAI-1), the major physiologic inhibitor of urokinase and TPA in plasma. This balance is maintained through processes that appear to be mediated largely by the endothelium. Plasminogen activators convert plasminogen to the active enzyme, plasmin, which is a protease that lyses fibrin clots. One important mechanism for regulating plasmin generation involves the formation of complexes between PAI-1 and the plasminogen activators, which prevents the conversion of plasminogen to plasmin.[1, 2] Because both TPA and PAI-1 are synthesized primarily by endothelial cells (and smooth muscle cells), the endothelium is thought to play a prominent role in maintaining vascular fibrinolytic balance.

Modest excesses or deficiencies in the fibrinolytic proteins can be associated with clinical consequences. Increased levels of PAI-1 have been associated with an increased risk of thrombosis in animal and clinical studies.[3–6] In one clinical study, low TPA activity and higher PAI-1 levels were observed in survivors of MI compared with healthy age-matched controls.[4] In another study, low TPA activity and increased PAI-1 concentrations were the only hemostatic variables associated with recurrent MI in a group of men with early coronary heart disease.[5] Imbalance of the fibrinolytic proteins can also have pathogenic consequences within the vascular wall. In vascular tissue, plasmin activates matrix metalloproteinases, which are crucial in remodeling following vascular injury through degradation of collagen and other glycoproteins that accumulate in plaques.[7] Several groups have reported increased deposition of PAI-1 in and around atherosclerotic plaques, which in turn reduces vascular plasmin activation and, subsequently, metalloproteinase activity. This reduction in plasmin activation is also associated with reduced activation of transforming growth factor-beta, which is important in suppressing the proliferation and migration of smooth muscle cells that contribute to atherosclerotic lesions.[8]

Regulatory Role of Endothelium in Fibrinolytic Balance and Role of the Renin-Angiotensin System

The role of the renin-angiotensin system (RAS) in regulating fibrinolysis was suggested by findings in two major clinical studies of angiotensin-converting enzyme (ACE) inhibitor therapy: the Survival and Ventricular Enlargement (SAVE) trial[9] and the Studies of Left Ventricular Dysfunction (SOLVD).[10] In both these studies, ACE inhibition significant-

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ly reduced the risk of recurrent MI and other ischemic events in patients with left ventricular dysfunction. In the SAVE trial, captopril treatment was associated with a 25% reduction in risk for recurrent MI and a 24% reduction in risk of death from cardiovascular events, severe heart failure, or MI. In SOLVD, which included more than 6,000 patients with asymptomatic left ventricular dysfunction or early congestive heart failure, enalapril was associated with an 18% reduction in risk of death from cardiovascular events and a 28% reduction in risk of death from MI.

The speculation that the endothelium serves as a link between the fibrinolytic system and the RAS has been supported by a number of experimental and clinical findings. Angiotensin II has been shown to bind to endothelial cells[11] and to stimulate dose-dependent production of PAI-1 in cultured rat vascular smooth muscle cells,[12] cultured bovine aortic cells,[13] and human endothelial cells,[13] thus demonstrating a potential link between the RAS and thrombosis. In other studies, ACE inhibition increased plasminogen activator activity in cultured bovine aortic endothelial cells[14] and decreased vascular PAI-1 expression in normal and balloon-injured rat aorta.[15] In human subjects, infusion of physiologic concentrations of angiotensin II resulted in rapid, dose-dependent, significant increases in PAI-1 levels (Fig. 1).[16] No significant changes in TPA levels were observed, indicating a selective effect of angiotensin II on PAI-1 release.

A recently identified angiotensin binding site, the angiotensin IV receptor (AT_4), appears to be the receptor on endothelial cells that mediates PAI-1 expression in response to angiotensin,[17] accounting for the observation that inhibitors of angiotensin receptors type 1 and type 2 fail to prevent endothelial production of PAI-1.[13, 17] The increase in PAI-1 expression in cultured cells is dependent on conversion of the octapeptide angiotensin to the hexapeptide angiotensin IV, which is accomplished via the effects of specific aminopeptides that are localized to the vascular surface.[17] Thus, responses to angiotensin may be mediated by an endothelial receptor specific for angiotensin IV, that is, the AT_4 receptor.

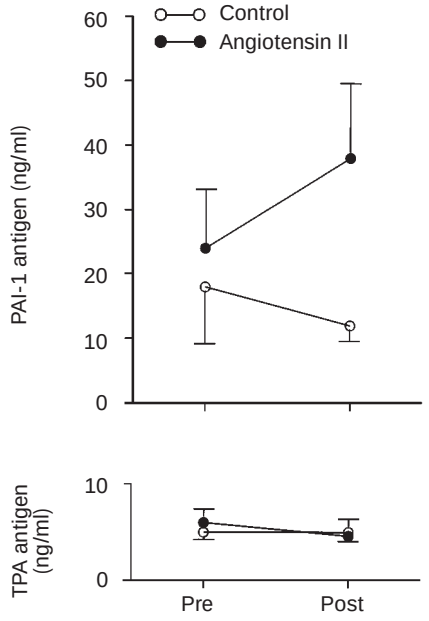

FIG. 1 Mean plasminogen activator inhibitor-1 (PAI-1) antigen levels and tissue-type plasminogen activator (TPA) antigen levels in hypertensive patients before and after infusion of angiotensin II (3 ng/kg/min for 45 min). Reprinted from Ref. No. 16 with permission.

Several clinical investigations, including recent studies designed to assess the effect of ACE inhibition on fibrinolytic factors, have provided additional evidence for the link between the RAS and the fibrinolytic system; these are reviewed below.

Angiotensin-Converting Enzyme and Fibrinolytic Balance

As suggested by the findings regarding ACE inhibition in cultured cells and in clinical studies, ACE occupies an important position in regulating the balance of fibrinolytic elements. It converts angiotensin I to angiotensin II, which is associated with stimulation of PAI-1 production. Through an independent and parallel pathway, ACE is also important in downregulating TPA production via degradation of bradykinin, a highly potent stimulator of TPA production in endothelial cells. In rats, intra-arterial administration of bradykinin results in a dose-dependent increase in plasma TPA levels.[18] In human subjects with hypertension, graded doses of bradykinin were associated with dose-dependent increases in plasma TPA levels during concomitant ACE inhibitor administration, but had no effect on TPA in the absence of ACE inhibitor administration (Fig. 2).[19] This finding confirms earlier reports that bradykinin is an extremely potent stimulus for the release of TPA in vivo. It also highlights the importance of the RAS in regulating vascular fibrinolytic balance.

Angiotensin-Converting Enzyme Inhibition and Fibrinolytic Balance

In addition to the experimental studies mentioned, clinical investigations have shown that ACE inhibition is associated with alterations in fibrinolysis. In the first study to demonstrate an effect of ACE inhibition on endogenous fibrinolysis, Wright *et al.*[20] administered captopril 75 mg/day or placebo to 15 patients beginning 8 weeks after uncomplicated MI and compared effects on fibrinolytic variables in these patients and 12 matched control subjects. The fibrinolytic variables assessed were PAI-1 antigen levels, TPA antigen levels, and PAI-1 activity. After the placebo treatment period, patients post MI had significantly higher TPA antigen and PAI-1 antigen levels and significantly greater PAI-1 activity than did controls. However, 4 weeks of ACE inhibition resulted in significant reductions in TPA antigen levels and PAI-1 activity in the 15 patients and a nonsignificant reduction in levels of PAI-1 antigen (Fig. 3).

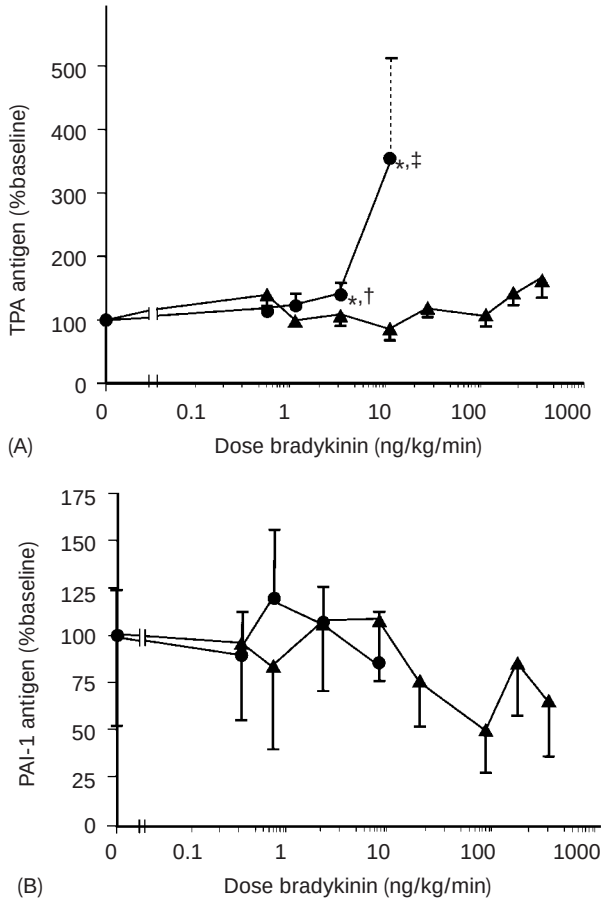

FIG. 2 Effect of bradykinin on (A) plasma TPA antigen levels and (B) levels of PAI-1 antigen in patients treated with either angiotensin-converting enzyme inhibitor (ACEI) or placebo. *$p<0.05$ versus baseline; †$p<0.05$ versus placebo; ‡$p<0.01$ versus placebo. ● = ACEI, ▲ = placebo. Reprinted from Ref. No. 19 with permission.

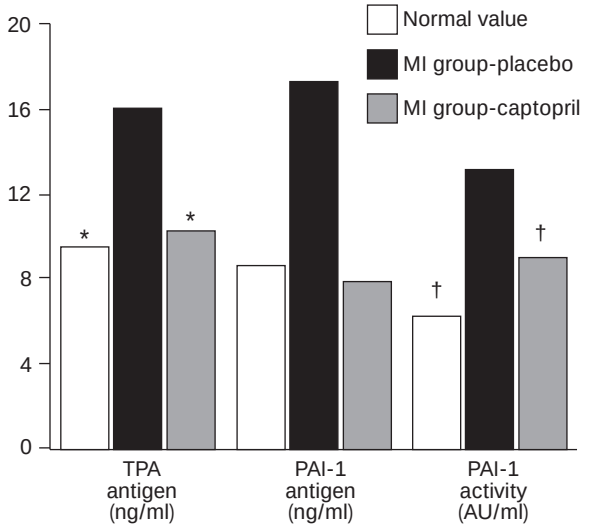

FIG. 3 Fibrinolytic variables in normal men and in patients with recent myocardial infarction (MI) after 4 weeks of placebo and 4 weeks of captopril. Adapted from data in Ref. No. 20 with permission.

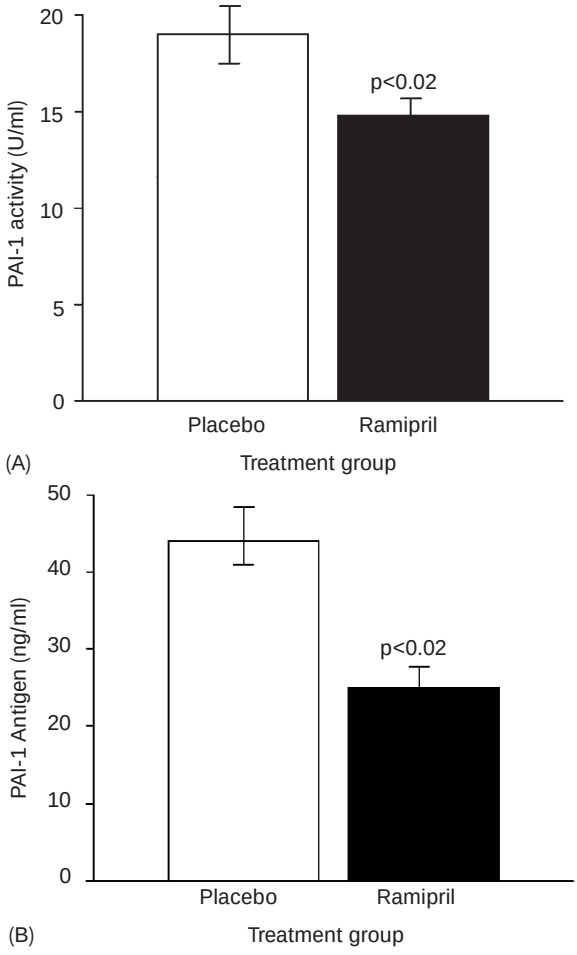

FIG. 4 Effects of ramipril and placebo on mean PAI-1 activity (A) and mean PAI-1 antigen levels (B) after 14 days of treatment. Reprinted from Ref. No. 21 with permission.

More recently, we[21] have assessed the effect of short-term ACE inhibition on fibrinolytic variables in a subset of 120 patients from the Healing and Early Afterload Reducing Therapy (HEART) study of patients with acute anterior MI and systolic blood pressure >100 mmHg. In this double-blind, placebo-controlled trial, patients were randomized to ramipril 0.625 or 1.25 mg/day titrated to 10 mg/day or placebo for 14 days. Subsequently, subjects in the placebo-treatment arm were crossed over into the high-dose ramipril arm of the study. Baseline PAI-1 activity and PAI-1 antigen and TPA antigen levels were comparable in the three groups; the ratio of PAI-1 to TPA, a measure intended as an index of fibrinolytic balance, was normal in each of the treatment groups as well. After 14 days, PAI-1 antigen levels were approximately 44% lower, and PAI-1 activity levels an average of 22% lower, in the patients treated with the ACE inhibitor (combined groups) than in placebo-treated patients (Fig. 4). In contrast, plasma TPA levels were not significantly different between the ACE inhibitor-treated patients and placebo-treated patients. Given

the significant reduction in PAI-1 activity and antigen levels, it is safe to say that ACE inhibition preserved normal fibrinolytic balance in these patients post MI. No other drugs besides ACE inhibitors have been shown to have such an impact on the plasma fibrinolytic balance during the recovery phase of acute MI. These results may help explain the beneficial effects of ACE inhibition on rates of MI and ischemic events in previous randomized trials.

Conclusion

There is accumulating evidence that the RAS interacts with the fibrinolytic system at the level of the endothelium. In fibrinolysis, both angiotensin II and ACE may be considered prothrombotic: angiotensin II because it induces PAI-1 expression, and ACE because it mediates the formation of angiotensin II and the degradation of bradykinin. Increased PAI-1 levels are associated with an increased risk of thrombotic events in humans. In experimental models, ACE inhibition is associated with reductions in PAI-1 expression in both cultured cells and tissue. These beneficial changes in fibrinolytic variables may be attributed to ACE inhibition's dual effects of inhibiting angiotensin II formation (and thus limiting the production of PAI-1) and blocking bradykinin degradation (and thereby enhancing the production of TPA by bradykinin). These mechanisms may contribute to the vasculoprotective effects of ACE inhibitors.

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