Brief Rapid Communication

Stimulation of Plasminogen Activator Inhibitor In Vivo by Infusion of Angiotensin II
Evidence of a Potential Interaction Between the Renin–Angiotensin System and Fibrinolytic Function

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Background. Recent clinical trial data indicate that the use of angiotensin converting enzyme (ACE) inhibitors among patients with left ventricular dysfunction results in reduced rates of coronary thrombosis, a provocative finding that suggests a potential interaction between the renin–angiotensin system and fibrinolytic function.

Methods and Results. In four normotensive subjects and six hypertensive patients, we investigated whether infusion of angiotensin II (Ang II) affected circulating levels of plasminogen activator inhibitor–1 (PAI-1), the most important physiological inhibitor of tissue-type plasminogen activator (t-PA). Overall, mean levels of PAI-1 antigen increased significantly from 20.1 ng/mL before Ang II infusion to 36.0 ng/mL at the end of Ang II infusion (p=0.008), whereas no change in PAI-1 was observed for control subjects infused with 5% dextrose (p=0.46). Among the normotensive subjects for whom graded doses of Ang II were infused at 0, 1, 3, and 10 ng·kg⁻¹·min⁻¹, mean PAI-1 levels increased sequentially from 14.7 ng/mL to 23.0, 26.8, and 33.5 ng/mL, a dose–response relation that, compared with controls, was highly significant (p<0.001). Among the hypertensive patients for whom a single 45-minute infusion of Ang II was given at a dose of 3 ng·kg⁻¹·min⁻¹, PAI-1 levels increased from 23.7 to 37.7 ng/mL, whereas PAI-1 levels among control subjects infused with 5% dextrose decreased from 16.9 to 10.8 ng/mL (p=0.04). Finally, when compared with infusion of 5% dextrose solution, infusion of Ang II appeared to have little effect on circulating levels of t-PA antigen.

Conclusions. These in vivo data suggest that infusion of Ang II results in a rapid increase in circulating levels of PAI-1, a finding that may help to explain clinical observations linking the renin–angiotensin system and thrombotic risk. (Circulation 1993;87:1969–1973)

Key Words • fibrinolysis • tissue-type plasminogen activator • plasminogen activator inhibitor–1 • thrombosis • angiotensin

Plasminogen activator inhibitor–1 (PAI-1) is the most important physiological inhibitor of tissue-type plasminogen activator (t-PA) in plasma, and elevated levels have been implicated in the pathogenesis of thromboembolic disease. Although the regulation of PAI-1 in vitro has been extensively studied, factors that control the production and secretion of PAI-1 in vivo are less well characterized.

Recently reported clinical trial data indicate that the administration of angiotensin converting enzyme (ACE) inhibitors to patients with left ventricular dysfunction following myocardial infarction results in reduced rates of recurrent coronary thrombosis, an observation that raises the possibility that angiotensin II (Ang II) may be involved directly in the thrombotic process. This observation is particularly intriguing as in vitro data indicate that Ang II selectively induces the production and secretion of PAI-1 in cultured astrocytes and endothelial cells and by the fact that elevated levels of PAI-1 appear to constitute a marker of risk for recurrent coronary thrombosis. Furthermore, prospective clinical data suggest that individuals with high renin essential hypertension are at a fivefold greater risk of sustaining myocardial infarction than individuals with low renin essential hypertension, although the mechanisms underlying this excess risk are unclear. Based on these observations, we hypothesized that in vivo infusion of Ang II might effect changes in circulating levels of PAI-1 in humans.

Methods

Subjects
Ten volunteers, four normotensive subjects and six patients with essential hypertension, were studied in the...
Clinical Research Center of the Brigham and Women's Hospital. All subjects were maintained on a low-salt diet containing 10 mmol Na and 100 mmol K for at least 5 days to achieve external sodium balance. Protocols for participation were approved by the Human Subjects Committee at the Brigham and Women's Hospital. Written and informed consent was obtained from all volunteers for the procedures. Secondary forms of hypertension were excluded by serum creatinine, urinalysis, plasma aldosterone, plasma norepinephrine and epinephrine, and 24-hour vanillylmandelic acid, norepinephrine, epinephrine, and hydroxycorticoid steroid levels. An additional four normotensive volunteers served as control subjects.

Ang II Infusions

When metabolic balance was achieved, subjects were fasted overnight. At 7:00 AM, intravenous lines were placed in each arm, one for infusion and one for blood sampling. Control samples were obtained, and an infusion of Ang II (Hypertensin, CIBA-GEIGY Co., Summit, N.J.) was administered. Similar procedures were followed for the control subjects, who were administered 5% dextrose solution instead of Ang II.

In study 1, which was designed to assess for evidence of a dose–response relation between Ang II and PAI-1 among the four normotensive volunteers, Ang II was infused at 1.0, 3.0, and 10.0 ng·kg⁻¹·min⁻¹ for 45 minutes at each dose level. To assess renal plasma flow, each volunteer also received an 8-mg/kg loading dose of para-aminohippuric acid (PAH) 60 minutes before starting Ang II followed by a constant rate of PAH infusion throughout the study period. Blood samples for PAI-1, t-PA, PAH, and cortisol were obtained at baseline and at the end of each dose of Ang II. Mean arterial blood pressure was monitored at 2-minute intervals using an indirect monitoring system throughout the Ang II infusion.

Study 2 was designed to assess the effect of Ang II infusion on PAI-1 and t-PA in six hypertensive volunteers who underwent a single 45-minute infusion of Ang II at 3.0 ng·kg⁻¹·min⁻¹. As in study 1, each volunteer also received an 8-mg/kg loading dose of PAH 60 minutes before starting Ang II followed by a constant rate of PAH infusion throughout the study period. Blood samples for PAI-1, t-PA, plasma renin activity, aldosterone, cortisol, and PAH were obtained at the start and end of the Ang II infusion. Blood pressure monitoring was performed throughout the infusion period.

Laboratory Analyses

All blood samples were placed on ice and immediately centrifuged. The separated plasma was frozen and stored at -70°C until the time of assay. Plasma samples were assayed for t-PA antigen and PAI-1 antigen using a two-site enzyme-linked immunosorbent assay (kits purchased from Biopool AB, Umeå, Sweden). Assays were performed in accordance with the manufacturer's instructions following a procedure described by Ranby et al.²³ Briefly, plasma samples were incubated in microtiter plates coated with monoclonal antibodies against the desired antigen, unbound antigens were washed off, and bound antigen was detected by addition of a second specific antibody conjugated to horseradish peroxidase. Standard curves were constructed using dilutions of purified antigen in plasma. The amount of t-PA and PAI-1 antigen in samples was deduced by comparing the sample absorbance with the calibration curve. In our laboratory, the coefficients of variation for repeated measures of t-PA antigen and PAI-1 antigen are 5.9% and 8.1%, respectively.

Aldosterone, cortisol, and plasma renin activity were measured by radioimmunoassay techniques that have been previously described.²²,²³ PAH concentration was measured by an autoanalyzer spectrophotometric technique,²⁴ and the clearance of PAH was calculated. The clearance of PAH represents about 90% of effective renal plasma flow when corrected for body surface area.

Statistical Analyses

Mean values for measured parameters were calculated at the start and end of each infusion, and the significance of any differences before and after infusion was determined using the paired Student's t test. In study 1, which assessed the dose–response effect of infusions on t-PA and PAI-1 antigens, a repeated-measures ANOVA was used to determine the significance of any difference between subjects receiving Ang II and subjects receiving 5% dextrose solution. All reported p values are two tailed.

Results

For all subjects receiving Ang II infusions, PAI-1 antigen levels increased from a mean of 20.1 ng/mL at baseline to 36.0 ng/mL at the end of the infusion period (p=0.008), whereas no significant change was observed among control subjects receiving 5% dextrose solution. In contrast, the t-PA antigen response to Ang II was heterogeneous with seven subjects demonstrating a decrease, two demonstrating no change, and one demonstrating an increase. Overall, mean t-PA antigen levels decreased from 7.9 ng/mL at baseline to 4.6 ng/mL at the end of Ang II infusion, which is a decrease of borderline statistical significance (p=0.06). The mean change in t-PA level among subjects infused with Ang II was not significantly different from the mean change in t-PA level observed among control subjects (−3.3 versus −0.4 ng/mL; p=0.26).

Figure 1 illustrates mean PAI-1 antigen and t-PA antigen levels for the four normotensive volunteers who received graded infusions of Ang II in study 1 and for the four normotensive controls who received 5% dextrose solution instead of Ang II. For subjects receiving Ang II, mean values of PAI-1 antigen increased with each increase in dose. Specifically, for these individuals, the mean preinfusion PAI-1 level of 14.7 ng/mL increased to 23.0, 26.8, and 33.5 ng/mL at the end of the 1.0-, 3.0-, and 10.0-ng·kg⁻¹·min⁻¹ infusions of Ang II, a dose–response effect that was highly significant compared with control subjects (p<0.001). Mean values of t-PA antigen decreased from 11.1 to 5.2 ng/mL over the graded infusion period, a dose–response effect that compared with controls was not statistically significant (p=0.10). As expected, Ang II infusion also resulted in an increase in mean arterial blood pressure from 83 to 96 mm Hg and a reduction in renal plasma flow from 614 to 417 mL·min⁻¹·1.73 m². Cortisol levels similarly decreased from 10.7 to 7.7 μg/dL.
Figure 1. Plots of mean plasminogen activator inhibitor–1 (PAI-1) and tissue-type plasminogen activator (t-PA) antigen levels at baseline and after increasing doses of angiotensin II among normotensive participants in study 1. Solid lines represent subjects receiving escalating doses of angiotensin II, while dotted lines represent control subjects receiving 5% dextrose solution. Error bars represent SEM.

Figure 2. Plots of mean plasminogen activator inhibitor–1 (PAI-1) and tissue-type plasminogen activator (t-PA) antigen levels before and after angiotensin II (A-II) infusion among the hypertensive participants in study 2. Solid lines represent subjects receiving a 45-minute infusion of A-II at a dosage of 3 ng·kg⁻¹·min⁻¹, whereas dotted lines represent control subjects receiving 5% dextrose solution. Error bars represent SEM.

Discussion

These in vivo data suggest that infusion of Ang II results in a rapid and significant increase in circulating levels of PAI-1 antigen. Among both normotensive and hypertensive subjects, this effect was apparent within 45 minutes of Ang II infusion and appeared to be related to the quantity of Ang II infused in a dose–response fashion. In contrast, infusion of Ang II led to a reduction in levels of t-PA antigen, although this decrease was small and of borderline statistical significance.

Taken together, these findings raise the possibility that Ang II may contribute to the development of a prothrombotic state at least in part by increasing plasma levels of PAI-1, thereby reducing the net activity of the endogenous fibrinolytic system. This potential relation between the renin–angiotensin system and fibrinolytic function may have important clinical and therapeutic consequences. Recently, two large-scale clinical trials have demonstrated that the administration of ACE inhibitors to patients with left ventricular dysfunction reduces the incidence of recurrent myocardial infarction by approximately 25%. The mechanism of this newly recognized effect of ACE inhibition is, however, unknown. For example, among the protean actions of ACE inhibitors are their ability to regulate bradykinin, which can increase the production of prostacyclin, endothelium-derived relaxing factor, and t-PA. If, as our data suggest, Ang II also effects a direct increase in PAI-1, then a further action of ACE inhibition may be to improve endogenous fibrinolytic function among patients at high risk for recurrent ischemic events. Such a potential relation between the renin–angiotensin system and endogenous fibrinolytic function may also help to explain the observation that patients with high renin essential hypertension (who presumably also have high Ang II levels) appear to be at greater risk for coronary thrombosis than do patients with similar levels of hypertension but lower plasma renin activity.

Although the current data represent the first in vivo demonstration that Ang II affects circulating PAI-1...
antigen levels, in vitro research from our laboratory indicates that Ang II induces the synthesis of PAI-1 in a dose-dependent fashion in cultured endothelial cells,\textsuperscript{17} a finding consistent with the recent report that Ang II selectively stimulates the production of PAI-1 in cultured murine astrocytes.\textsuperscript{16} Thus, these data strongly suggest that Ang II be added to the list of agents known to enhance endothelial PAI-1 synthesis and secretion, factors that include endotoxin,\textsuperscript{4-6} interleukin 1,\textsuperscript{7} tumor necrosis factor,\textsuperscript{8,9} transforming growth factor-\(\beta\),\textsuperscript{9} insulin and insulinlike growth factors,\textsuperscript{10-12} and the glucocorticoid hormones.\textsuperscript{13} In addition, because no pressor effects are generated in cell culture preparations, these in vitro findings from disparate cell sources make it highly unlikely that the PAI-1 response to Ang II is dependent on any hemodynamic stimulation or blood pressure response. In fact, prior in vivo and in vitro research from several groups consistently indicate that pressor infusions lead to increases in t-PA with either no change in PAI-1 or a concomitant decrease in PAI-1.\textsuperscript{26-29} Thus, the current finding that Ang II infusion leads to an increase in PAI-1 but not t-PA strongly suggests that the mechanism of the observed Ang II effect is not pressor related.

Despite the concordance of our in vivo Ang II data with those observed for Ang II in vitro,\textsuperscript{10,17} three potential limitations of the current study must be considered. First, because PAI-1 levels demonstrate a diurnal variation in normal subjects, it theoretically is possible that the time- and dose-related effects we observed occurred independent of Ang II infusion. However, in contrast to the subjects receiving Ang II, the control subjects demonstrated a decline in PAI-1 antigen over time. This finding is consistent with previous research describing the circadian variation of PAI-1, which has demonstrated that levels peak in the early morning hours (when our baseline samples were obtained) and then progressively decrease over the course of the day, a pattern that, if anything, would dilute any true Ang II-induced increase.\textsuperscript{30,31} Second, because infusion of Ang II leads to a reduction in renal plasma flow, it is possible that the increases in PAI-1 we observed with Ang II infusion resulted solely from reduced renal clearance. This explanation for our observed effect is also unlikely as the primary metabolic pathway for PAI-1 is hepatic rather than renal. Moreover, if reduced renal clearance is the sole mechanism leading to increased PAI-1 levels, then t-PA levels would also be expected to increase with Ang II infusion. In the present study, however, levels of t-PA antigen increased in only one of 10 subjects after Ang II infusion. Finally, because increases in glucocorticoid hormone levels have been shown to elevate PAI-1,\textsuperscript{13} it is possible that an increase in cortisol could contribute to the increase in PAI-1. However, cortisol levels fell in our study during Ang II infusion, following the usual circadian rhythm.

In summary, these data suggest that infusion of Ang II into normal as well as hypertensive subjects results in a rapid and substantial increase in circulating levels of PAI-1 without a significant change in t-PA. These findings may help to explain clinical observations that suggest a potentially important relation between the renin–angiotensin system and thrombotic risk. Further studies with larger sample sizes will be needed to assess whether a difference exists between normotensive and hypertensive subjects with regard to the PAI-1 response to Ang II.

References

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