Coagulation and Inhibitory and Fibrinolytic Proteins in Essential Hypertension


ABSTRACT
Arterial hypertension (HTN) increases the risk of cerebral, coronary, and other vascular complications that frequently involve platelet activation and blood coagulation. Several key proteins in the blood coagulation, fibrinolytic and inhibitory systems were studied in 29 men with HTN (aged 45 ± 3 yr) and 15 normal men of the same age. Plasma levels of high-molecular-weight kininogen and factors XII, IX, VII, X, II, and XIII, as well as von Willebrand factor (vWF), fibrinogen, fibronectin, α2-antiplasmin, tissue-plasminogen activator, D-dimer, platelet factor-4, and protein C were measured by the use of appropriate functional and immunologic assays before and after a cardiopulmonary exercise stress test. The concentrations of vWF, α2-antiplasmin, and D-dimer were significantly (P < 0.02) higher in the HTN group as compared with the control group. The exercise stress test resulted in significant rises in the plasma vWF, α2-antiplasmin, and tissue-plasminogen activator levels in the two groups. The concentrations of vWF and D-dimer were related to diastolic blood pressure (r = 0.44 and 0.40, respectively; P < 0.02). Levels of vWF also were related to left ventricular mass index and left ventricular posterior wall and septal thickness (r = 0.34, 0.43, and 0.34, respectively; P < 0.05). The constellation of these findings suggests a low-grade fibrin formation and degradation, the magnitude of which is related to the diastolic blood pressure. The observed abnormalities can potentially contribute to the cardiovascular complications of untreated HTN.

Key Words: Hypertension, coagulation system, fibrinolytic system, protease inhibitors, left ventricular mass

Platelet activation and blood coagulation play a major role in the genesis of cerebral, coronary, and peripheral vascular complications. In this regard, hyperfibrinogenemia and elevated plasma factor VII have been identified as possible independent risk factors for ischemic cardiovascular disease (1–4). In view of the strong association between hypertension (HTN) and ischemic cardiovascular complications, studies of blood coagulation and related systems appear to be relevant in hypertensive populations. Published data on the state of the blood coagulation, fibrinolytic, and inhibitory systems in patients with arterial HTN are limited. In this study, plasma activity or the concentration of various key proteins in the coagulation, fibrinolytic, and inhibitory pathways was assessed in a group of patients with essential HTN and a group of normal volunteers of the same sex and comparable age.

PATIENTS, MATERIALS, AND METHODS

Patients
Twenty-nine men with essential HTN aged 45 ± 13 (SD) yr (range, 26 to 71 yr) were included in the study (Table 1). They represented consecutive patients who presented to the HTN center and consented to participate in the study.

The participants were free of all cardiovascular disease except for uncomplicated, mild-to-moderate essential HTN. The patients were either newly diagnosed as being hypertensive or had been off of anti-hypertensive medications for at least 3 months before enrollment in the study. Arterial blood pressure (BP) was determined by conventional sphygmomanometry at each of three separate clinical visits. Individuals were allowed at least 3 min of rest in the seated position before two BP measurements, taken a minute apart, were determined by use of the first and fifth phase Korotkov sounds. BP measurements from the three visits were averaged, and subjects with a mean diastolic BP greater than 90 mm Hg were considered hypertensive.
Patients with secondary HTN, cardiovascular complications, liver disease, renal insufficiency, proteinuria, primary coagulopathy, and/or acute intercurrent illnesses and those receiving drugs known to affect blood coagulation system were excluded.

Normal Control Group

A group of 15 healthy normotensive men aged 40.3 ± 16 yr (range, 19 to 71 yr) served as the control group. They were studied contemporaneously with the HTN group and had no evidence or history of renal disease, liver disease, coagulopathy, or use of any drugs. Clinical characteristics of the patients and controls are shown in Table 1, and except for blood pressure, there were no significant differences between the two groups.

Cardiopulmonary Exercise Stress Testing

Cardiopulmonary exercise stress testing was carried out by a modification of the Balke-Ware protocol. All patients were encouraged to exercise to their maximum ability. During each test, the electrocardiogram was continually monitored and recorded at 1-min intervals with a Case 12 Electrocardiographic System (Marquette Electronics Inc., Milwaukee, WI). Physical exercise is known to stimulate the release of certain endothelial-derived proteins such as tissue-plasminogen activator (t-PA) and von Willebrand factor (vWF). The exercise tests were carried out to compare the response in the study populations.

Blood Samples

Free-flowing venous blood samples were obtained from an antecubital vein after a 30-min resting period between the hours of 8:30 and 9:30 a.m. The subjects then underwent cardiopulmonary exercise stress testing. A second blood sample was obtained immediately after the completion of the exercise test. Samples were withdrawn with plastic syringes and plastic tubes containing 3.8% sodium citrate (blood/citrate = 9/1 vol). The citrate-anticoagulated blood was immediately centrifuged, and plasma was separated and stored at -80°C until assayed.

Samples for platelet factor-4 (PF4) determination were obtained as follows. Free-flowing venous blood was withdrawn and immediately placed in chilled tubes containing a citrate/citric acid mixture supplemented with theophylline, adenosine, and dipyrindamide to inhibit platelet aggregation. The tubes were then placed in an ice-water bath and allowed to cool for 15 min. The samples were then centrifuged at 2,500 × g and 2 to 8°C for 30 min. Platelet-poor plasma was harvested by pipetting the middle-third portion of the plasma away from the top surface, which may contain light platelets, and away from the platelet layer above the cell layer.

Materials and Methods

The following factor-deficient plasma substrates were purchased from Dade Diagnostics Inc. (Miami, FL): congenital human factor XII-deficient plasma, congenital human factor VII-deficient plasma, and factor II-deficient substrate prepared from aged human serum and barium sulfate-adsorbed bovine plasma.

The antisera against human high-molecular-weight kininogen and factor XII were purchased from ICN Inc. (Lisle, IL). Antiserum against human factor IX was purchased from American Bioproducts ( Parsippany, NJ), and antisera to human factor II, protein C, α₂-antiplasmin, fibronectin, and factor XIII a-subunit were purchased from Calbiochem Inc. (La Jolla, CA). Coagulation assay reference plasma purchased from Helena Laboratories (Beaumont, TX) served as the normal pooled plasma standard.

Supplies for the determination of the antigen concentrations of the following proteins were purchased from American Bioproducts: vWF, factor VII, factor X, D-dimer, t-PA, and PF4. Fibrinogen concentration was determined by the method of Clauss (5) with a kit purchased from Sigma Chemical Co. (St. Louis, MO). Supplies for protein C amidolytic activity determination were purchased from American Diagnostics (Greenwich, CT).

Functional Assays

The procoagulant activity of factor XII was determined in a one-stage clotting assay (modification of partial thromboplastin time), and the plasma coagulant activities of factors VII and II were determined.
in a two-stage clotting assay (modifications of Quick time). Congenitally or otherwise, factor-deficient plasmas were used as substrates in the above assays. The intra-assay coefficients of variation of the assays for factors XII, VII, and II activity were 3.8, 3.2, and 2.9%, respectively.

Antiplasmin activity was determined by an amidolytic method with plasmin excess and a chromogenic substrate in which the amount of plasmin activity inhibited is proportional to the amount of antiplasmin present in the test sample. The intra-assay coefficient of variation for this assay was 2.2%.

Protein C amidolytic activity was measured by the use of a specific snake venom activator and a synthetic chromogenic substrate. The intra-assay coefficient of variation for this assay was 2.2%.

All assays were conducted in duplicates, and the average of the two values was used. The assays for each of the proteins studied were carried out in a single session to avoid interassay variations. A standard curve was constructed with each assay with multiple dilutions of the normal pooled plasma standard. The activity of each protein in the test sample was determined by interpolation of the results into the appropriate standard curve.

**Immunnoassays**

Antigen concentrations of high-molecular-weight kininogen, factor XII, factor IX, factor II, a2-antiplasmin, protein C, fibronectin, and factor XIII a-subunit were determined by immunoelectrophoresis with monospecific antibodies produced in goat or rabbit against respective purified human proteins. The intra-assay coefficients of variation for the above assays were 3.4, 4.1, 4.3, 3.9, 3.5, 4.0, and 2.8% respectively.

Plasma vWF, factor VII, factor X, D-dimer, t-PA, and PF4 were quantitated by an enzyme-linked immunosorbent technique by the use of a double-antibody sandwich method. The intra-assay coefficients of variation for the above assays were 2.5, 1.9, 3.7, 2.7, 4.2, and 5.4%, respectively.

A standard curve was constructed for each assay session with multiple dilutions of the given reference standard. The antigen concentration of each protein in the test sample was determined by interpolation of the result into the appropriate standard curve.

**Urine Collection**

A 24-h urine collection was obtained to determine the creatinine clearance and to test for albumin excretion before the exercise study. The albumin concentration in the urine was measured by the use of rate immunonephelometry as described previously (6).

**Echocardiography**

M-mode and two-dimensional echocardiograms were done within 1 wk of the study session. Recordings were from standard parasternal and apical windows with a phased-array echocardiograph (General Electric, Pass II or RT500, Milwaukee, WI). M-mode left ventricular (LV) measurements were determined according to the recommendations of the American Society of Echocardiography (7).

**Statistical Analysis**

Statistical comparisons involving two groups of unpaired data were made by the use of two-tailed unpaired t tests with combined or separate variance estimates of the t statistic as indicated by an F test. A paired t test was used to compare data obtained before and after exercise. Correlation coefficients were determined by Pearson's method. P values less than 0.05 were considered significant. The results of the coagulation and related proteins are expressed as a percentage of the corresponding values found in the normal pooled plasma standard unless otherwise indicated. Data are given as mean ± SE unless otherwise noted.

**RESULTS**

**Baseline Values**

Data are shown in Tables 2 through 4. The plasma antigen concentration of vWF was significantly higher (P < 0.02) in the HTN group when compared with that found in normal controls. Likewise, the HTN group showed a significant increase in plasma concentrations of D-dimer (P < 0.02) and a2-antiplasmin (P < 0.01) and a decrease in antiplasmin activity (P < 0.01) compared with the control values. This was accompanied by a significant increase in plasma protein C concentration. Although the mean plasma fibrinogen concentration in the HTN group was greater than that found in the control group, the difference did not reach statistical significance. No significant difference was found in the plasma procoagulant activities of factors XII, VII, and II in the HTN group when compared with those observed in the controls. Likewise, plasma antigen concentrations of factors XII, IX, VII, X, II, and XIII and those of high-molecular-weight kininogen, fibronectin, t-PA, and PF4 were not significantly different in the two groups.

Although mean urinary albumin excretion rate in the HTN group (11.9 ± 6 mg/day) was greater than that found in the control group (3.8 ± 0.5 mg/day), the difference did not attain statistical significance.
TABLE 2. Comparison of plasma antigen concentrations of high-molecular-weight kininogen factor XII, factor IX, and vWF and factor XII procoagulant activity in hypertensive and normal control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>High-Molecular-Weight Kininogen Antigen Conc (%)</th>
<th>Factor XII Antigen (%)</th>
<th>Factor XII Activity (%)</th>
<th>Factor IX Antigen Conc (%)</th>
<th>vWF Antigen Conc (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td>99 ± 6.5</td>
<td>100 ± 6.0</td>
<td>92 ± 6.8</td>
<td>88 ± 3.1</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>Normotensive</td>
<td>82 ± 6.0</td>
<td>107 ± 5.3</td>
<td>85 ± 7.0</td>
<td>79 ± 3.7</td>
<td>6.7 ± 0.7</td>
</tr>
</tbody>
</table>

P Value NS

*NS, nonsignificant. Conc, concentration.

TABLE 3. Plasma concentrations of factors VII, X, II (prothrombin), clottable fibrinogen, Factor XIII, and fibronectin and activities of factors VII and II in hypertensive patients and normotensive controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Factor VII Antigen Conc (%)</th>
<th>Factor VII Activity (%)</th>
<th>Factor X Antigen Conc (%)</th>
<th>Factor II Antigen Conc (%)</th>
<th>Factor II Activity (%)</th>
<th>Fibrinogen Conc (mg/dL)</th>
<th>Factor III Antigen Conc (%)</th>
<th>Fibronectin Antigen Conc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td>86 ± 2.4</td>
<td>104 ± 10.9</td>
<td>89 ± 2.0</td>
<td>101 ± 5.7</td>
<td>103 ± 3.2</td>
<td>222 ± 9.5</td>
<td>115 ± 7.8</td>
<td>80 ± 7.4</td>
</tr>
<tr>
<td>Normotensive</td>
<td>82 ± 2.2</td>
<td>96 ± 5.9</td>
<td>87 ± 4.2</td>
<td>94 ± 8.8</td>
<td>102 ± 4.1</td>
<td>209 ± 15.6</td>
<td>112 ± 9.8</td>
<td>68 ± 7.7</td>
</tr>
</tbody>
</table>

P Values 0.05

*P values were all nonsignificant. Conc, concentration.

TABLE 4. Plasma antigen concentrations of protein C, α2-antiplasmin, t-PA, D-dimer, and PF4 and protein C and plasma antiplasmin activity in hypertensive and normotensive groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein C</th>
<th>Antiplasmin Activity (%)</th>
<th>α2-Antiplasmin Conc (%)</th>
<th>Antiplasmin/Conc Ratio (%)</th>
<th>t-PA Antigen Conc (ng/mL)</th>
<th>α-dimer Conc (ng/mL)</th>
<th>PF4 Antigen Conc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td>107 ± 8.3</td>
<td>119 ± 6.8</td>
<td>1.19 ± 0.07</td>
<td>95 ± 5.6</td>
<td>0.92 ± 0.08</td>
<td>5.8 ± 0.6</td>
<td>159 ± 23.8</td>
</tr>
<tr>
<td>Normotensive</td>
<td>85 ± 5.0</td>
<td>105 ± 5.2</td>
<td>1.31 ± 0.10</td>
<td>74 ± 4.2</td>
<td>1.31 ± 0.06</td>
<td>5.0 ± 0.9</td>
<td>82 ± 18.5</td>
</tr>
</tbody>
</table>

P Values 0.05

*NS, nonsignificant. Conc, concentration.

Effect of Exercise

With exercise, the diastolic BP fell by 5.0 ± 1.4 mm Hg in the HTN group and by 4.9 ± 2.3 mm Hg in the control group. There was no significant difference in the magnitude of the exercise-induced fall in diastolic BP between the two groups (P = not significant). After exercise, hematocrit, hemoglobin, and plasma concentrations of total protein and albumin rose significantly in both groups. No significant difference was noted in the extent of exercise-induced changes in these parameters between the HTN and control groups. Likewise, vWF, α2-antiplasmin, D-dimer and t-PA concentrations rose significantly in all subjects and the magnitude of the relative increase was similar for both groups. Of note, the magnitude of the relative exercise-induced rises in PF4, vWF, and t-PA levels was significantly (P < 0.05) greater than that observed with either serum albumin, total protein, or erythrocyte count (Table 5).

Association With BP and Echocardiographic Data

The relationship between preexercise diastolic BP and preexercise vWF and D-dimer concentrations are illustrated in Figures 1 and 2. There were significant positive associations between the preexercise diastolic BP and the preexercise plasma concentrations...
TABLE 5. Comparison of relative exercise-induced change in vWF, t-PA, PF₄, red blood cell count, and total protein in hypertensive and normal control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>vWF Antigen % Change</th>
<th>t-PA Antigen % Change</th>
<th>PF₄ Antigen % Change</th>
<th>Red Blood Cell Count % Change</th>
<th>Total Protein % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td>60 ± 14.1</td>
<td>195 ± 44</td>
<td>107 ± 39</td>
<td>5.3 ± 1.1</td>
<td>12.1 ± 1.4</td>
</tr>
<tr>
<td>Normotensive</td>
<td>49 ± 14.0</td>
<td>150 ± 23</td>
<td>58 ± 22</td>
<td>8.8 ± 2.7</td>
<td>12.6 ± 1.5</td>
</tr>
</tbody>
</table>

* Values are given as percent change relative to the preexercise measurements (postexercise – preexercise/postexercise x 100). No significant differences were found between hypertensive and normotensive groups. P values were all nonsignificant.

Echocardiographic Findings

There was no significant difference between the two groups with respect to LV mass index, posterior wall or septal thickness, and LV end-diastolic or end-systolic dimensions.

DISCUSSION

The results revealed a significant elevation of vWF antigen concentration at resting conditions in patients with essential HTN. This multimeric macromolecular glycoprotein is produced by the endothelial cells and plays a major role in platelet aggregation and thrombus formation (8). A transient elevation of vWF occurs in response to a variety of acute insults and stresses and to the administration of vasopressin or adrenergic agents (9, 10). The marked rise in plasma vWF after the exercise stress test, demonstrated here, clearly reflects this transitory phenomenon. In addition, persistent elevation of vWF has been demonstrated in such prothrombotic states as
nephrotic syndrome (11,12), unstable angina pectoris (K. Lehmann and N.D. Vaziri, unpublished data), and acute myocardial infarction (13). Accordingly, the observed elevation of resting plasma vWF in patients with HTN may contribute to a heightened state of platelet reactivity and blood coagulability.

The elevation of resting plasma vWF in hypertensive patients is unlikely to be due to its reduced consumption rate. On the contrary, the concurrent elevation of D-dimer, which indicates low-grade activation of the coagulation and fibrinolytic systems, points to the increased consumption of the hemostatic factors. Accordingly, increased plasma vWF concentration is most likely due to its augmented production and release in the HTN group. It is conceivable that the increase in the concentration of this endothelial-derived hemostatic factor may reflect the cardiovascular stress associated with elevated BP. This viewpoint is supported by the positive correlation observed between diastolic BP and vWF concentration. A similar conclusion can be drawn from the significant correlations observed with LV mass index and the thickness of the LV posterior wall and interventricular septum, which probably are affected by the elevated arterial BP.

The hypertensive group showed a marked elevation of plasma D-dimer concentration under resting conditions, indicating heightened fibrin formation and degradation. The observed elevation of plasma D-dimer in the absence of a clinically discernible thromboembolism in the hypertensive patients suggests low-grade activation of the coagulation system and thrombin formation coupled with concurrent action of the fibrinolytic and inhibitory systems. In addition to promoting fibrin formation, thrombin serves as a potent activator of platelets (14). It is of note that both thrombin and products of platelet activation have been shown to trigger growth-related signals that lead to hypertrophy and mitogenesis in smooth muscle preparations (15–17). It should be noted that both free and endothelial-bound thrombin molecules are readily inactivated by antithrombin and thrombomodulin, respectively. However, fibrin-bound thrombin is refractory to these inhibitory influences and tends to remain active at the site of clot formation (1). Such persistent thrombin activity at the site of fibrin deposition can potentially promote cellular proliferation in the vessel wall and contribute to the development and progression of arteriosclerosis.

The hypertensive group showed a significant increase in plasma a2-antiplasmin antigen concentration relative to that found in the normal control group. a2-Antiplasmin is the most potent inhibitor of plasmin and, as such, possesses a strong antifibrinolytic property. Consequently, abnormal elevation of this protein can contribute to a prothrombotic state. It is of interest that elevation of a2-antiplasmin antigen concentration was associated with a reduced antiplasmin activity. This phenomenon could have been due to a reduction in the activity of other plasmin inhibitors such as a2-macroglobulin. However, this is an unlikely possibility because these inhibitors play a minor role in plasmin inactivation. Alternatively, this may be due to the presence of an inactive or plasmin-bound molecule, which, although retaining immunoreactivity, lacks functional activity. A third explanation may be an increased proportion of the less-active nonplasminogen-binding isoform relative to the potent plasminogen-binding isoform of a2-antiplasmin (18) in the HTN group as compared with that in the control group. Recently, Gleerup and Winther reported a marked impairment of fibrinolytic activity as measured by euglobulin clot lysis time in a group of patients with untreated hypertension (19). This was associated with a marked elevation of plasminogen activator inhibitor (PAI) and a reduction in t-PA activity. They attributed the impairment of fibrinolytic activity and t-PA function to the abnormal elevation of PAI. Our finding of increased a2-antiplasmin reveals an additional mechanism for the abnormal fibrinolytic activity in patients with untreated HTN at a more distal point within the fibrinolytic cascade. I.e., the inhibition of plasmin itself. Interestingly, the basal t-PA antigen concentration in our HTN patients was not depressed. This observation supports the interpretation of Gleerup and Winther of reduced t-PA activity being due to elevated levels of its inhibitor, I.e., PAI as opposed to a low concentration of t-PA.

The mean plasma protein C level in the hypertensive group was greater than that in the normal controls. It should be noted that the activated protein C serves as a potent inhibitor of the activated forms of the two important nonenzymatic cofactors in the coagulation cascade, I.e., factors VIII and Va, and, as such, represents an important, naturally occurring anticoagulant. In addition, by inactivating PAI-1, activated protein C promotes fibrinolysis (20). Consequently, the elevation of protein C in our HTN patients may provide some measure of protection in a condition that is otherwise marked by hypercoagulability and impaired thrombolysis.

Plasma concentrations of vWF, t-PA, D-dimer, and a2-antiplasmin rose significantly after exercise tests in both groups. The magnitude of the relative rises in vWF and t-PA concentrations was much greater than that seen with either serum total protein, albumin, or erythrocyte concentration, which merely reflected exercise-induced fluid shift to the extravascular space. These observations suggest the active production/secretion of both vWF and t-PA from the vascular endothelium during exercise. In addition, PF4 concentration increased with exercise in both groups. Although the magnitude of the rise in PF4,
was greater in the HTN group, the difference did not attain statistical significance. Nonetheless, this observation may indicate a tendency for greater stress-mediated release of this potent heparin inhibitor from the platelets in the HTN group. The trend observed here is consistent with several previous reports that demonstrated increased platelet reactivity, aggregation, and adhesion in patients with HTN (21–24). The magnitude of the exercise-induced relative rise in vWF was insignificantly greater in the HTN group as compared with that in the normal controls, indicating the possibility that HTN may exaggerate vascular stress associated with heavy exercise. In addition, the exercise test helped to reveal significant correlations between certain coagulation-related proteins and the BP and LV dimensions.

In conclusion, the study presented here has provided a comprehensive profile of blood coagulation and related systems in men with relatively mild and predominantly diastolic HTN. The results indicate that untreated HTN is associated with an elevation of endothelium-derived vWF and a low-grade activation of the coagulation and fibrinolytic systems. The extent of the observed changes is related to the diastolic BP and the thickness of the LV wall and interventricular septum. These observations point to the relationship between untreated HTN, the hemostatic system, and target organ effects.

REFERENCES