Mechanism and Clinical Significance of the Prothrombotic State in Patients With Essential Hypertension

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ABSTRACT

Background: Thrombotic, rather than hemorrhagic, events represent a major complication of hypertension. This study aims to explore the mechanism of the hypercoagulative state in hypertension and to assess its clinical significance.

Hypothesis: The hypercoagulative state and even the prothrombotic state exists in patients with hypertension. This may be attributed to an impairment of the endothelium.

Methods: A total of 81 patients suffering from essential hypertension were classified into 3 groups (grade 1: n = 27; grade 2: n = 36; grade 3: n = 18) and an additional 28 nonhypertensive patients were used as the control group. This study determined the changes of platelet activation marker P-selectin (CD62P), plasma fibrinogen, plasminogen activitor inhibitor-1 (PAI-1), and endothelium function.

Results: The percentage of CD62P+ platelets and the concentration of plasma fibrinogen and PAI-1 in the hypertension group was significantly higher than those in the control group. These increments coincided with the elevation of blood pressure. A significant difference was found between any of the 2 hypertension subgroups in the percentages of CD62P+ platelets ($P < 0.001$) and the concentration of PAI-I ($P < 0.05$). No difference was noted between the hypertension grade 1 and 2 groups in the concentration of plasma fibrinogen ($P = 0.079$); however, a significant difference was found between any of the other 2 subgroups ($P < 0.001$). Flow-mediated dilation (FMD) in the hypertension group was significantly lower than that in the control group.

Conclusions: The hypercoagulative state exists in patients with hypertension and this state was more obvious with the elevation of blood pressure and coincided with an impairment in the degree of endothelium-dependent vasodilation.

Introduction

Despite the exposure of blood vessels to high pressures, the literature has reported that thrombotic, rather than hemorrhagic, events represent a major complication of hypertension,[1–3] indicating that the hypercoagulative and even prothrombotic state accompanies hypertension; this paradoxical thrombotic[3] phenomenon has garnered increasing attention. Previous literature[4–7] generally detected some clotting factors and the links between the various systems reported little. In this study, we measured the changes of platelet activation marker P-selectin (CD62P), plasma fibrinogen (FIB), plasiminogen activitor inhibitor-1 (PAI-1), and endothelium function simultaneously. We also discussed the interactions of different systems, stressing the importance of endothelial injury and platelet activation in its contribution to the prothrombotic state. The vessel wall, blood constituents, and the blood stream are main factors in the process of thrombogenesis and are intimately related to endothelial function. When the endothelium is impaired, blood liquidity, blood coagulation, and vasomotion are no longer well-regulated. Another difference from my traders is that we detected the nitroglycerin-mediated dilation (NMD), as well as the flow-mediated dilation (FMD), because though vascular smooth muscle changes may exist in hypertension, it can still be compensatory and not sufficiently influence vasomotion. Instead, the vasomotion is influenced by endogenous substances produced by impairment of the endothelium, therefore it is more convenient to say that the endothelial injury exists in hypertension. Why is an endothelial injury so important to the prothrombotic state forming? How do multiple systems interact? The elaborate mechanisms of the hypercoagulative state and its clinical significance will be discussed further.

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Methods

Study Patients

A total of 109 patients between 18 and 80 years of age were enrolled: 81 with essential hypertension (grade 1: n = 27; grade 2: n = 36; grade 3: n = 18) and 28 as the control group. The exclusion criteria included: secondary hypertension; aggravated damages to the heart, brain, liver, kidney, and blood; and diabetes mellitus. General clinical conditions such as age, gender, body mass index, smoking, drinking, hyperlipidemia, family history of hypertension, and coronary heart disease were comparable between groups (see Table 1). The nature, purpose, and potential risks of the study were carefully explained to each subject before informed consent was obtained. The study protocol was in accordance with the Declaration of Helsinki.

Study Content

All antihypertensive medications, except for diuretics, in the experimental group were stopped for 1 week. The use of aspirin or other drugs that interfere with platelet function was prohibited for 2 weeks.

Venous peripheral blood was collected from all patients between 6 and 7 AM on the first day of admission. The percentages of CD62P+ platelets, and FIB, and the concentration of FIB were measured by flow cytometry (Z2/Z1s/Z1d, Beckman Coulter, Inc, USA), automatic blood coagulation analysis meter (ACL-200, Beckman Coulter, Inc, USA), and enzyme-linked immunosorbent assay (ELISA; Shanghai Sun Biological Technology Co Ltd, Shanghas, China), respectively, according to standard procedures.

Noninvasive, high-resolution ultrasound (ACUSONSE-QUOIA 512, made in USA) was used to detect the dilation changes of brachial arteries during reactive hyperemia and after sublingual nitroglycerin.

Study Method

CD62P+ determination: The first milliliter of the 2 mL blood sample was discarded, while the remainder was anticoagulated with 2% EDTA, 5 μL of which was mixed with 20 μL of CD62P+ marked by phycoerythrin (PE). The mixture was shielded from light at room temperature for 20 minutes, then immediately mixed with 1 mL of 1% paraformaldehyde precooled at 2–8°C, then stored in a 2–8°C refrigerator for 2 hours for the final test by flow cytometry within 24 hours, the blank being the control. The scattered light intensity and 3-color fluorescence intensity of each platelet in both the experimental and control groups were measured, as well as, the percentage of CD62P+ platelets and mean fluorescence intensity (MFI). The MFI ratios measured in the experimental and control group test tubes represented the relative amount of platelet activation marks.

FIB determination: After the blood sample was collected and anticoagulated, 2 mL was delivered to the Centralab of the China-Japan Union Hospital of Jilin University. The concentration of FIB was determined by an automatic blood coagulation analysis meter in the central lab.

PAI determination: The blood sample was anticoagulated with 2% EDTA, centrifuged at 3000 r/mi for 10 minutes and the supernatant liquid stored in a 2–8°C refrigerator for a maximum period of 48 hours. When the test began, the liquid was first placed in 37°C water baths for 15 minutes, diluted to one-tenth of the original concentration followed by resuspending in a 0.3 mL diluent to produce 120 ng/mL. A total of 150 μL of this solution was taken to produce

Table 1. Baseline Characteristics of Hypertensive and Control Groups

Variable	Hypertension (n = 81)	Control (n = 28)	*P* Value
Age (yrs)	68.50 ± 5.12	58.30 ± 4.56	>0.05
Sex (M/F)	53/28	17/11	>0.05
Body mass index (kg/m²)	23.80 ± 3.01	24.01 ± 2.73	>0.05
Smoking/nonsmoking	50/31	12/16	>0.05
Drinking/nondrinking	38/43	12/16	>0.05
Hypertension family history	47	12	>0.05
Triglyceride (mmol/L)	3.34 ± 1.36	1.01 ± 0.31	<0.05
Total cholesterol (mmol/L)	4.07 ± 0.79	3.94 ± 0.82	>0.05
High-density lipoprotein cholesterol (mmol/L)	1.07 ± 0.38	1.24 ± 0.08	>0.05
Low-density lipoprotein cholesterol (mmol/L)	5.56 ± 1.50	2.44 ± 0.47	<0.05
Coronary heart disease	41	11	>0.05

120, 60, 30, 15, 7.5, 3.75, 1.875 ng/mL standard solutions through multiple dilution. 100 μL of each standard and sample was respectively put into the ELISA kit 37°C water baths for 150 minutes. Washed, dried and added enzyme labeled antibodies 100 μL each in 37°C water baths for 60 minutes, washed and dried as before, developed with o-pheylenediamine (OPD), termination, measured at the 490 nm from the microplate reader model 550 BIO-RAO, USA. The double logarithmic chart about A490 and the concentration of PAI-1 was then plotted and we got the concentration of PAI-1 of sample from the curve.

Assessment of Endothelial Function

To assess endothelial function, the Celermajer method[8] was followed. First, the patient rested in a supine position for 10 minutes in a quiet place, followed by a determination of the shape and diameter of the brachial artery (D_0) on the right arm 10 centimeters above the elbow. After banding the sphygmomanometer cuff to produce a pressure of 50 mm Hg higher than the systolic pressure for 5 minutes, then released the gas the brachial artery diameter (D_1) was measured 60 seconds after degassing. The patient was then instructed to rest for approximately 15 minutes until the brachial artery diameter returned to normal, and then 0.5 mg of nitroglycerin was taken sublingually; 3 minutes later, the brachial artery diameter (D_2), the flow-mediated dilation ($FMD = [\{D_1 - D_0\}/D_0] \times 100\%$), and the nitroglycerin-mediated dilation of brachial arteries ($NMD = [\{D_2 - D_0\}/D_0] \times 100\%$) were detected.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 10.0, SPSS Inc, Chicago, IL, USA). Normally distributed data were described as mean ± standard deviation and statistical analysis was performed by independent samples *t* test. Non-normally distributed data were presented as median (first to third interquartile range) and was analyzed by the Kruskal-Wallis test. Categoric data were analyzed by the χ^2 test. A probability of $P < 0.05$ was considered to be statistically significant.

Results

Comparison of the Hypertension and the Control Group

The percentages of CD62P+ platelets, concentration of FIB, and PAI-1 in the hypertension group were significantly higher than those in the control group ($60.82\% \pm 9.74\%$ vs $47.84\% \pm 10.01\%$ CD62P+ platelets, $P < 0.001$; 2.91 ± 0.60 vs 2.61 ± 0.35 g/L FIB, $P = 0.005$; 70.16 ± 4.28 vs 39.99 ± 19.85 ng/mL in PAI-I, $P < 0.001$).

Comparison of Hypertension Subgroups

The percentages of CD62P+ platelets in grade 1, 2, and 3 groups were $52.14\% \pm 6.80\%$, $61.23\% \pm 5.68\%$, and $67.87\% \pm 8.68\%$, respectively, while that of the control group was $47.84\% \pm 10.01\%$. A significant difference was found between any other of the 2 hypertension subgroups ($P < 0.001$).

The concentration of FIB in grade 1, 2, and 3 groups was 2.45 ± 0.47, 2.58 ± 0.37, and 3.36 ± 0.51 g/L, respectively, while the control group was 2.61 ± 0.35 g/L. Little difference was noted between the hypertension grade 1 and 2 groups ($P = 0.079$); however, a statistical difference was found between any other of the 2 subgroups ($P < 0.001$).

The concentration of PAI-1 in grade 1, 2, and 3 groups was 54.30 ± 5.68, 67.02 ± 2.75, and 81.44 ± 4.17 ng/mL, respectively, while the control was 39.99 ± 19.85 ng/mL. The difference was significant between any other of the 2 hypertension subgroups ($P < 0.05$).

Variation Trend of CD62P+, FIB, and PAI-1 With Blood Pressure

From Figures 1 through 3, we can see that the percentages of CD62P+ and the concentration of FIB and PAI-1 showed obvious increases with blood pressure.

FMD and NMD

The basic diameter (D_0) of brachial arteries in hypertension was 3.58 ± 0.52 mm compared with the value of

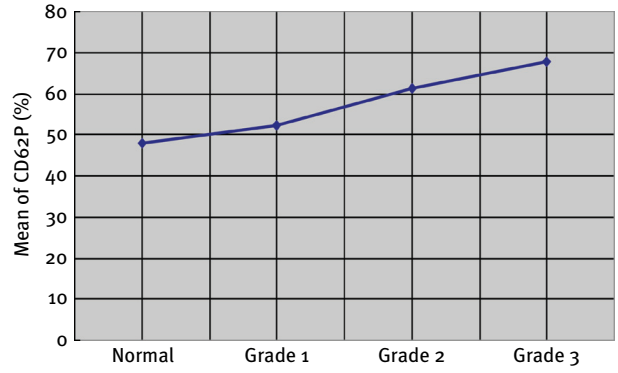

Figure 1. Mean of platelet activation marker P-selectin (CD62P).

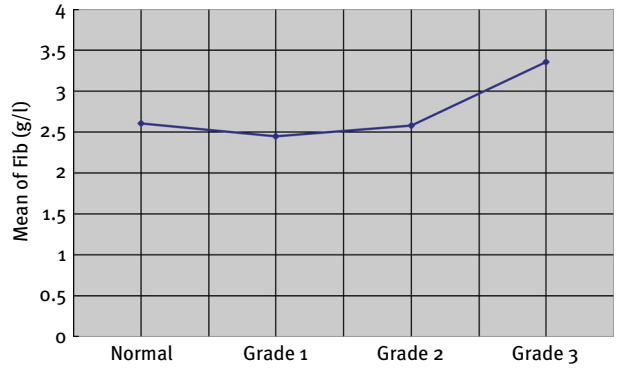

Figure 2. Mean of plasma fibrinogen (FIB).

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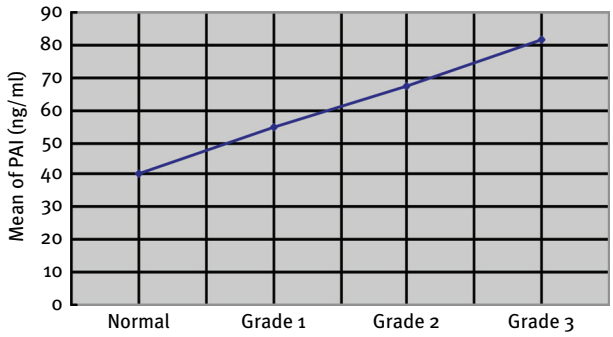

Figure 3. Mean of plasminogen activitor inhibitor-1 (PAI-1).

3.59 ± 0.38 mm for the control group; no significant difference was found between these 2 groups. The brachial arteries diameter of FMD (D_1) in the hypertension and control groups was 3.84 ± 0.54 mm and 4.23 ± 0.43 mm, respectively, and the diameter of NMD (D_2) was 4.23 ± 0.64 mm and 4.40 ± 0.43 mm, respectively. Flow-mediated dilation in the hypertension group was significantly lower than the controls (7.26% ± 2.87% vs 17.82% ± 2.67%, $P < 0.05$). No difference was found between the hypertension and control group in the NMD of the brachial arteries (18.25% ± 5.84% vs 22.56% ± 4.15%, $P < 0.05$; see Table 2).

Discussion

Thrombotic events after hypertension have high morbidity and mortality, so exploring its mechanism and the early intervention of the prothrombotic status are of clinical significance.

Under physiological conditions, blood fluidity is maintained by the normal function of platelets and the balance of hemostatic and fibrinolytic factors. CD62P+, FIB, and PAI-1 are the most specific markers of the activation of the platelets, coagulation, and fibrinolysis system, respectively, reflecting the status of coagulation and fibrinolysis. Increased CD62P+, FIB, and PAI-1 indicate that the coagulation system is activated and that the fibrinolytic system is inhibited, which makes the blood clot easily and more liable to induce thrombotic diseases. The results of our study show that not only were there increases in CD62P+, FIB, and PAI-1 in patients with essential hypertension, but that they were made more obvious with the elevation of blood pressure, indicating that a hypercoagulated status exists in hypertensive patients, and that this prothrombolytic status is influenced by the level of hypertension.

Injured endothelial cells and an activated renin-angiotensin-aldosterone system (RAAS) accompanied by high blood pressure played pivotal roles in the regulation of the hypercoagulable status and in the promotion of thrombosis after hypertension.[9]

Vascular endothelial cells have the function of endocrine, autocrine, and paracrine: not only do they regulate the vascular tone through the release of some vasoactive substances, such as nitric oxide (NO), endothelin, prostacycline, thromboxane A2, and angiotensin, they also participate in platelet activation, leukocyte adhesion, and the regulation of thrombosis, and so forth.

There are 2 kinds of vasodilations, namely endothelium-dependent and non-endothelium-dependent. Flow-mediated dilation is a reflection index of endothelium-dependent vasodilation: after the brachial artery was occluded by the compression of the cuff and transient ischemia and anoxia were created, the damaged endothelial cells released an endothelium-derived relaxing factor such as NO, thus leading to vasodilation. Nitroglycerin-mediated dilation, on the other hand, is a reflection index of non-endothelium-dependent vasodilation: vascular smooth muscle-relaxing drugs such as nitroglycerin and sodium nitroprusside have direct effects on the vascular smooth muscle, increase the cyclic guanosine monophosphate, and then cause vasodilation. Under normal circumstances, diameter changes induced by these 2 kinds of vasodilation are similar. In this study, the change of the endothelium-dependent vasodilation is smaller than that of non-endothelium-dependent vasodilation, indicating impaired endothelial function in patients with hypertension. Following the endothelium impairment and the degranulation of activated thrombocytes, CD62P+ in the cytoplasma of resting thrombocytes is combined with the platelet membrane and therefore expresses as a marker of platelet activation.

Table 2. FMD and NMD in Hypertensive and Control Group

Group	n	D_0 (mm)	D_1 (mm)	D_2 (mm)	FMD (%)	NMD (%)
Hypertensive	81	3.58 ± 0.52	3.84 ± 0.54	4.23 ± 0.64	7.26 ± 2.87[a]	18.25 ± 5.84
Control	28	3.59 ± 0.38	4.23 ± 0.43	4.40 ± 0.43	17.82 ± 2.67	22.56 ± 4.15

Abbreviations: FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation.
[a] There is statistical significance ($P < 0.05$)
Do: Brachial Artery Basic Diameter
D1: Brachial Artery Diameter of FMD
D2: Brachial Artery Basic Diameter of NMD.

The elevation of CD62P+ in this study indicated the high activity of platelet function. These highly activated platelets adhere to the collagen of the damaged endothelium, gather and upon further activation results in the formation of platelet thrombus. On the surface of a formed platelet thrombus, streptokinase reaction of clotting factors occurs, followed by the transformation of fibrinogen into fibrin.[10] Recently, it has been confirmed that an unstable blood stream was the primary contributing factor; under the blood flow-associated shear stress, many vasoactive substances, including NO, an endothelial derived relaxing factor, were released by the endothelium,[11,12] which results in the reduction of platelet adhesion and the relaxation of vascular smooth muscle.[13] Reduced NO bioactivity is a major component of endothelial dysfunction.[14] In this study, FMD in the hypertension group was significantly lower than those in the control group, indicating the impairment of endothelium-dependent vasodilation in hypertension, decreased sensitivity of endothelium to stimulating factors, reduced release of NO secretion, and the greater production of tissue factor (TF) and PAI-1 with the impaired endothelium cells.[15,16] In this study, the increment of CD62P+ and PAI-1 might affect the blood flow, at the same time the endothelium itself might be affected by changes of blood contents and the pressure of the blood flow.[15,16] The change of hemodynamics activated the endothelium-producing substances such as PAI-1, the most important regulatory factors in the fibrinolysis system, released mainly from the endothelium and platelets and adjusted by angiotensin-II and aldosterone. Increased PAI-1 indicates that the fibrinolytic system is inhibited and blood is in a hypercoagulable state. The abnormalities in fibrinolysis led to endothelial damage and dysfunction.[17] Increased PAI-1 has been considered a hallmark of endothelial dysfunction and this dose-dependent PAI-1 promotes the formation of endothelial microparticles via reducing the transmembrane asymmetry of phospholipids.[18] The more unstable blood flow with elevated blood pressure results in its susceptibility to induce thrombotic diseases.

The prothrombotic/hypercoagulable state can also be induced by the activated renin-angiotensin system.[19] The RAAS reduced fibrinolysis by increasing PAI-1 expression.[20,21] In consensus with previous publications that the elevated PAI-1 levels are associated with target organ damages in subjects with newly diagnosed arterial hypertension,[22] some studies[23] have suggested that angiotensin-converting enzyme increases the production of the components of the extracellular matrix, such as FIB, by adjusting the angiotensin-II receptors. In our study, the elevation of PAI-1 and FIB indicate that the prothrombotic state is closely related to the activation of the RAAS in patients with hypertension.

The elevations of CD62P, FIB, PAI-1, and FMD in patients with hypertension confirmed the activation of coagulation/fibrinolytic, endothelial, and RAAS, which contributed to the formation of a prothrombotic status. Antihypertension drugs with the capability of reversing the impaired endothelium function and inhibiting RAAS will have more beneficial effects on the patients with essential hypertension.

Conclusions

In this article, first, we highlighted the importance of endothelial injury and platelet activation in their contributions to the prothrombotic state. Endothelial injury exposes tissue factors and may activate the extrinsic coagulation factor to produce thrombin; however, this is not sufficient to activate the fibrin. A large number of platelets activated by an impaired endothelium would be necessary in order to provide a platform for thrombosis. Compared to the coagulation/fibrinolytic system, platelet activation plays a greater role in coagulation. Second, endothelial-injury activated RAAS interacting with other systems eventually led to the prothrombotic state. Third, the medication of hypertension should be good to emphasize the treatments improving the endothelial function besides antiplatelet and anti-renin-angiotensin system. Only by a reasonable clinical application of drugs to improve or reverse the endothelial function will it be adequate in controlling the prothrombotic state in patients with essential hypertension.

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