Increased Plasma Levels of Platelet-Derived Growth Factor (PDGF-BB + PDGF-AB) in Patients With Never-Treated Mild Essential Hypertension

Ermanno Rossi, Bruno Casali, Giuseppe Regolisti, Simona Davoli, Franco Perazzoli, Aurelio Negro, Carlo Sani, Bruno Tumiati, and Davide Nicoli

Platelet-derived growth factor (PDGF) could play a role in both vascular hypertrophy and atherosclerotic disease associated with hypertension. To assess whether plasma PDGF level is increased in mild essential hypertension, we measured plasma PDGF concentration in 25 never-treated patients with uncomplicated mild essential hypertension and in 22 normotensive healthy subjects. To evaluate the contribution of platelets to plasma PDGF in the two groups, we also measured plasma β-thromboglobulin (BTG). Measurement of PDGF was carried out through an enzyme-linked immunoadsorbent assay, which detects two PDGF dimers, namely PDGF-BB and PDGF-AB. Both plasma PDGF and BTG were higher in the hypertensive than in the normotensive subjects. The ratio of PDGF to BTG was similar in the two groups. Plasma PDGF was weakly correlated with plasma BTG in the normotensive subjects, whereas this relationship was lost in the hypertensive patients. Our results suggest that the increase in plasma PDGF (PDGF-AB + PDGF-BB) in never-treated essential hypertension is mainly due to platelet activation. The increased circulating level of PDGF could play a role in the vascular structural changes associated with hypertension. Am J Hypertens 1998; 11:1239–1243 © 1998 American Journal of Hypertension, Ltd.

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Essential hypertension is associated with increased stiffness of the large arteries and narrowing of the resistance vessels. Evidence from animal models suggests that these abnormalities are due to arterial structural changes, partly consisting of an increase in smooth muscle mass and connective tissue in the media. In spontaneously hypertensive rats, hypertrophy associated with an increase in smooth muscle cell (SMC) ploidy has been observed in the large arteries, whereas SMC hyperplasia, characterized by an increased number of SMC, has been shown in resistance vessels. These structural changes of the vessel wall may play a fundamental role in the maintenance and amplification of hypertension, and possibly in the acceleration of atherosclerosis associated with hypertension. The mechanisms underlying hypertensive vascular growth have not yet been determined, but evidence is accumulating that both mechanical and...
circulating or autocrine/paracrine humoral factors contribute to the arterial growth in hypertension. Among the humoral factors, platelet-derived growth factor (PDGF) has also been implied.

Initially regarded as platelet specific, PDGF is also produced by vascular SMC, endothelial cells, monocytes/macrophages, and fibroblasts.\(^6,7\) PDGF is composed of two homologous polypeptide chains (A and B), and can occur in all three possible dimers (namely, PDGF-AA, PDGF-BB, and PDGF-AB).\(^6\) Platelets, macrophages, and endothelial cells can produce both PDGF chains, whereas vascular SMC produce only PDGF-A chains.\(^6,7\) PDGF receptors (PDGFR), which belong to the receptor-tyrosine kinase superfamily, consist of combinations of two different subunits, namely PDGFR-\(\alpha\) and PDGFR-\(\beta\). PDGFR-\(\alpha\) can bind both PDGF-A and PDGF-B chains, whereas PDGFR-\(\beta\) binds only the PDGF-B chain.\(^8\) PDGF binding to SMC surface PDGFR generates a complex network of intracellular signal pathways, eventually leading to several possible effects on SMC, including mitogenesis, chemotaxis and migration, contraction, and synthesis of matrix proteins.\(^7\)

In vascular SMC of animal models of hypertension, increased expression of PDGF-A chain mRNA,\(^8\) PDGFR-\(\beta\) mRNA,\(^9\) and of both PDGF-\(\alpha\) mRNA and PDGFR-\(\alpha\) protein\(^10\) has been reported. Moreover, the growth effects of some vasoconstrictor substances involved in hypertension, such as angiotensin II and catecholamines, seem to be partly mediated by an increased expression of both PDGF-A chain\(^11,12\) and PDGFR-\(\beta\)\(^13\) in SMC. Furthermore, an increased expression of PDGF-B chain has been shown in endothelial cells from stroke-prone spontaneously hypertensive rats.\(^14\) Altogether these findings suggest a possible role for autocrine/paracrine secretion of PDGF by arterial wall cells in mediating SMC hypertrophy/hyperplasia associated with hypertension.

The PDGF plasma level probably reflects the release rate of the substance by the cells able to produce it, including platelets, in addition to vessel wall cells. In platelets, PDGF is stored in the \(\alpha\) granules together with platelet-specific proteins, such as \(\beta\)-thromboglobulin (BTG) and platelet factor 4 (PF-4). These substances are coreleased in the platelet release reaction.\(^15\) Therefore, an increase in plasma PDGF associated with a similar change in plasma BTG will mainly reflect a state of platelet activation.

In this study we investigated whether plasma PDGF is increased in never-treated mild essential hypertension and whether it parallels plasma BTG level.

**MATERIALS AND METHODS**

**Subjects** Twenty-five recently diagnosed and never treated patients with mild, uncomplicated essential hypertension and 22 normotensive healthy subjects participated in the study. The two groups were well matched for age, gender, body mass index, smoking habits, serum creatinine, platelet count, and plasma levels of glucose, total cholesterol, and triglycerides (Table 1).

Individual blood pressures were taken as the average of six office measurement in the seated position on three different visits. Blood pressure was measured with a standard mercury sphygmomanometer, using Korotkoff phase V for diastolic blood pressure. Mild hypertension was defined by diastolic blood pressure levels between 90 and 105 mm Hg, or systolic blood pressure levels between 140 and 180 mm Hg. Normotension was defined by blood pressure levels less than 135/85 mm Hg. The diagnosis of essential hypertension was established after exclusion of secondary hypertension on the basis of appropriate clinical and laboratory evaluation. Cardiovascular complications were ruled out by physical examination and electrocardiography. In all subjects other acute or chronic diseases were excluded by standard clinical and laboratory examinations. No subject had taken drugs affecting platelet function for at least 1 month before the study.

All subjects gave their informed consent; the protocol was approved by the local ethics committee.

**Sample Preparation** To avoid ex vivo platelet activation, blood samples for plasma PDGF and BTG determinations were drawn from an antecubital vein with a 21-gauge needle, without venous occlusion, and immediately collected in prechilled tubes contain-

| TABLE 1. CHARACTERISTICS OF THE STUDY GROUPS |
|-------------------------------|------------------|------------------|
|                              | Hypertensive  \((n = 25)\) | Normotensive \((n = 22)\) |
| Age (years)                  | 46 ± 8          | 45 ± 7           |
| Gender (M/F)                 | 15/10           | 13/9             |
| Body mass index (kg/m\(^2\)) | 23.7 ± 1.6      | 24.1 ± 1.2       |
| SBP (mm Hg)                  | 147 ± 11        | 119 ± 5*         |
| DBP (mm Hg)                  | 97 ± 6          | 77 ± 5*          |
| Smokers/nonsmokers           | 8/17            | 7/15             |
| GLucose (mg/dL)              | 88.3 ± 18.2     | 91.3 ± 12.8      |
| Total cholesterol (mg/dL)    | 205.5 ± 40.8    | 199.4 ± 35.8     |
| Triglycerides (mg/dL)        | 106.5 ± 47.5    | 97.7 ± 35.5      |
| Serum creatinine (mg/dL)     | 1.01 ± 0.16     | 0.99 ± 0.13      |
| Platelet count \((\times 10^9/\mu L)\) | 236.53 ± 58.28 | 229.25 ± 39.42 |

\(^*P < .0001.\)
ing sodium citrate, theophylline, adenosine, and dipyridamole (Diatube H, Diagnostica Stago, Asnieres, France). Immediately after collection, blood samples were centrifuged at 4500 g for 15 min in a Sorvall RC-5 centrifuge precooled at 4°C. Platelet-poor plasma supernatant (PPPS) was carefully collected in 1.5-mL polystyrene tubes (Eppendorf, Hamburg, Germany) and stored at −70°C until assay. All specimens were assayed in the same run.

PDGF Assay  An enzyme-linked immunosorbent assay (ELISA) was developed following the method of Dunbar et al., with slight modifications. One IgG1 monoclonal antibody to PDGF B-chain (PGF-007, Mochida Pharmaceutical, Tokyo, Japan) and one goat polyclonal antiPDGF antibody reacting with both the A- and B-chains (Collaborative Research, Boston, MA) were used; therefore two PDGF dimers, namely AB and BB, could be detected by this assay. As a first step, plastic microtiter plates (International PBI, Milan, Italy) were coated overnight with PGF-007 5 μg/mL in carbonate buffer at 4°C, and then blocked with 200 μL of PBST buffer (0.05% Tween-20, phosphate-buffered saline and 0.5% gelatin) at 22°C for at least 1 h. One hundred microliters of PDGF standard (Sigma Chemical Co., St Louis, MO) diluted in PBST or 100 μL of undiluted PPPS were then added in duplicate and incubated overnight at 4°C. Subsequently, goat antimouse PDGF at 20 μg/mL was incubated at 4°C for 5 h, followed by 1/250 (v/v) rabbit antigoat IgG (Dako, Glostrup, Denmark) at 22°C for 1 h. Goat peroxidase-antiperoxidase complex (Sigma Chemical Co.) at 1/250 dilution was then incubated at 22°C for 1 h, followed by O-phenylenediamine with 0.01% hydrogen peroxide at 22°C for 10 min. The reaction was stopped with 2 mol/L sulphuric acid, and absorbance read within 10 min at 492 nm on an automated ELISA plate reader (AutoReader III, Ortho Diagnostic System, Raritan, NJ). Linear regression analysis performed on the standard curve yielded a correlation coefficient exceeding 0.98. The coefficient of variation was less than 8% within an assay and less than 14% between assays. The detection limit was 0.15 ng/mL.

β-Thromboglobulin Assay  BTG was measured in diluted (1/10, v/v) plasma samples using a commercial immunoassay kit (Asserachrom BTG, Boehringer Mannheim, Germany). For each subject, the same plasma aliquot was used for both PDGF and BTG assays.

Statistics  After verifying the normality of the data distribution, differences in the mean of continuous variables were analyzed with Student’s t test for unpaired data, whereas χ² was used for categorical variables. Linear regression was used to explore the relationship between PDGF and BTG plasma levels.

RESULTS
The characteristics of the hypertensive patients and control normotensive subjects are shown in Table 1. Plasma levels of PDGF (PGF-AB + PGF-BB) and BTG in the two groups are presented in Figure 1. Both plasma PDGF (0.63 ± 0.23 ng/mL vs 0.45 ± 0.14 ng/mL; P = .013) and BTG (17.11 ± 6.25 IU/mL vs 12.66 ± 3.24 IU/mL; P = .007) were higher in the hypertensive than in the normotensive subjects.

The ratio of PDGF (ng/mL) to BTG (IU/mL) in the former group was not significantly higher than that in the controls (0.041 ± 0.024 vs 0.037 ± 0.013; P = .38). However, plasma PDGF was weakly though significantly (r = 0.4, P = .03) correlated with plasma BTG in the normotensive, whereas this relationship was lost in the hypertensive subjects (r = −0.1, P = .28).

DISCUSSION
The results of this study suggest that the increase in PDGF plasma level (PGF-AB + PGF-BB) in never-treated mild essential hypertension is mainly due to platelet activation. In fact, both PDGF and BTG plasma levels were increased in hypertensives, with a PDGF/BTG ratio not significantly greater than that in the controls. This is consistent with previous studies showing platelet abnormalities in hypertensives. Indeed, an increase in intracellular Ca²⁺ concentration, plasma level of BTG, thrombin-stimulated platelet adhesion, and platelet expression of P-selectin has been reported in patients with essential hypertension. Furthermore, platelet extracts obtained from hypertensive patients present an enhanced growth-promoting activity on cultured vascular SMC. Nevertheless, the absence of a significant correlation between plasma PDGF and BTG in the hypertensive patients, in contrast with normotensive subjects, may support the hypothesis of other cell sources, possibly cell components of the arterial wall, contributing to the increased plasma PDGF. This assumption is consistent with the increased expression of PDGF-A and PDGF-B in the arterial wall of animal models of hypertension. Whatever the source may be, an increased level of plasma PDGF could play a role in the vascular structural changes associated with hypertension. Indeed, the effects of growth factors on the arterial wall could be mediated even by their circulating levels, in addition to their autocrine/paracrine pathways. In fact, in rats with coartation hypertension, an increased wall thickness unrelated to sympathoadrenergic influence has been shown even in protected normotensive arterioles, which suggests the involvement of circulating humoral growth factors. Moreover, the growth effects of increased plasma PDGF levels could be en-
hanced by the upregulation of PDGF receptors in hypertensive vessels.9,10

In conclusion, the altered platelet function together with the increased circulating PDGF level could play a role in the acceleration of atherosclerosis associated with hypertension.

REFERENCES


