Inhibition of Tissue Factor Pathway During Intermittent Pneumatic Compression
A Possible Mechanism for Antithrombotic Effect

Vibhuti D. Chouhan, Anthony J. Comerota, Ling Sun, Russell Harada, John P. Gaughan, A. Koneti Rao

Abstract—Intermittent pneumatic compression (IPC) devices are an effective prophylaxis against lower extremity deep vein thrombosis. Their antithrombotic effect has been attributed to a reduction in venous stasis and enhanced fibrinolysis. The initiating mechanism for blood coagulation is the tissue factor (TF) dependent pathway, which is inhibited by tissue factor pathway inhibitor (TFPI). We have investigated the effect of IPC on the TF pathway in 6 normal subjects and 6 patients with postthrombotic venous disease undergoing IPC for 120 minutes; all subjects were studied with each of 5 IPC devices. In normal subjects and patients, plasma factor VIIa (FVIIa) activity (the activated form of factor VII [FVII]) declined from mean values ranging 51 to 65 and 50 to 53 mU/mL before IPC with different devices to 10 to 13 and 20 to 22 mU/mL at 180 minutes, respectively (P<0.001 for all groups). FVII antigen levels were unchanged. Plasma TFPI (P<0.001) rose from mean baseline values ranging 69 to 79 and 57 to 61 ng/mL to 76 to 123 and 71 to 79 ng/mL at 180 minutes in normal subjects and patients, respectively (P<0.001 for all groups). Plasma prothrombin fragment F1.2 levels showed minimal changes. There was an inverse relationship between TFPI and FVIIa in normal subjects (r=−0.31, P<0.001) and patients (r=−0.37, P<0.001). IPC results in an increase in plasma TFPI and decline in FVIIa. Inhibition of TF pathway, the initiating mechanism of blood coagulation, is a possible mechanism for the antithrombotic effect of IPC. (Arterioscler Thromb Vasc Biol. 1999;19:2812-2817.)

Key Words: intermittent pneumatic compression ■ tissue factor pathway ■ factor VIIa ■ tissue factor pathway inhibitor ■ factor VII

Intermittent pneumatic compression (IPC) is an established nonpharmacological method of prophylaxis against deep vein thrombosis.1 The mechanisms for the antithrombotic effects of IPC are unclear and generally considered to be due to reduction of venous stasis in the lower extremities and stimulation of endogenous fibrinolytic mechanisms.1–4 However, the effects of IPC on the early events of blood coagulation are unknown. The initiating mechanism of blood coagulation is the tissue factor (TF)–dependent pathway, which is regulated primarily by the tissue factor pathway inhibitor (TFPI), a Kunitz type inhibitor circulating in plasma.5–7 This pathway is initiated when factor VIIa (FVIIa), the activated form of FVII, is exposed to its cofactor, tissue factor (TF), leading to formation of TF-FVIIa complex, which proteolytically activates factor X (FX) and factor IX (FIX).5–7 TFPI inhibits FXa directly and the TF-FVIIa complex in the presence of FXa.5–8 Thus TFPI is a key natural anticoagulant that functions at the initial steps of blood coagulation.5–7 Although a thrombotic disease secondary to an inherited TFPI deficiency is yet to be recognized, there is evidence in rabbits suggesting that TFPI protects against thrombosis after exposure of blood to TF9,10 and decreases mortality in Escherichia coli–induced shock in baboons.11 Plasma TFPI levels are elevated after intravenous administration of heparin.12,13 It has been suggested that TFPI contributes to the anticoagulant properties of heparin14 and that heparin-releasable pool of TFPI may be of physiological importance.13 Plasma TFPI levels increase after exercise15 and in several disease states.13

Because of the increasing recognition of TFPI as a major modulator of the TF pathway and the existence of TFPI pools that can be potentially mobilized, we postulated that the antithrombotic effects of IPC may be related to modulation of the TF-mediated pathway of blood coagulation. In this study, we report our findings in 6 normal subjects and 6 patients with a history of venous thrombotic disease subjected to IPC using 5 separate devices.
Methods
Six normal subjects (4 males, 2 females; mean age 46 years, range 25 to 68 years) and 6 patients (4 males, 2 females; mean age 48 years, range, 31 to 66 years) with postthrombotic chronic venous insufficiency were studied during IPC with each of 5 different devices. The patients had a history of proximal deep vein thrombosis and evidence of recanalization and thickened venous walls on venous duplex studies. They were matched by sex and age to the normal subjects. Two of the patients were on oral anticoagulants at the time of the studies. These patients were studied to determine whether presence of underlying chronic venous disease impacts on the response to IPC.

Blood samples were also obtained from 3 normal subjects who were handled in an identical manner to the study subjects but were not subjected to IPC. All studies were performed after approval by the institutional review board and each participant signed an informed consent.

Devices
Five external compression devices were studied in random sequence; these included thigh-length sequential compression sleeves (TSQ, SCD Kendall Healthcare Products Company); calf-length sequential compression sleeves (CSQ, SCD Kendall Healthcare Products Company); thigh length single chamber garments DVT30 (TSC, Flotronics Huntleigh Healthcare); calf length single chamber garments (CSC, Flotronics Huntleigh Health Care); and Plexipulse foot pump (FP, NuTech). Two of these are applied to the calf region (CSQ, CSC), 2 extend to the thigh region (TSC, TSG), and 1 (FP) is applied only to the foot. Each volunteer rested in a supine position for at least 15 minutes before studies with each device. The devices were applied for 120 minutes as per manufacturer’s instructions.

Each volunteer was studied at the same time of day with an interval of at least 7 days between studies. Blood samples were drawn from antecubital vein, without application of a tourniquet, into one-tenth volume of 3.8% sodium citrate. They were obtained at baseline, 60, 120, and 180 minutes after start of IPC. Plasma was harvested by centrifugation at 2500g for 20 minutes within 30 minutes of collection. Plasma was stored as aliquots at −80°C.

Assays
Factor VIIa was measured using recombinant soluble tissue factor with a commercially available kit (Diagnostica Stago).16 FVII antigen levels were measured using an enzyme linked immunosassay (Diagnostica Stago) and expressed as a percentage of the value in pooled normal plasma. TFPI antigen was measured using Immunobind total TFPI ELISA kit from American Diagnostica Inc.17 This assay measures both free TFPI, lipoprotein associated TFPI, and complexes of TFPI with FXa and TF-FVIIa. Prothrombin fragment F1.2 levels were measured using an ELISA (Behring Diagnostic Inc). Antithrombin activity was measured using a chromogenic substrate S-2238 (Pharmacia Hepar) and bovine thrombin (Armour Pharmaceutical Co).18 The antithrombin activity is expressed as a percent of activity in pooled normal plasma.

Statistical Analysis
Data are presented as means±standard error of mean. Data from each plasma protein measurements were analyzed separately using a logarithmic transformation of the data. A 3-factor factorial design was used with repeated measures on the third factor, the sample time. The factors were as follows: treatment groups (normals and patients), intermittent compression devices (5 different ones), and sample times (0, 60, 120, and 180 minutes). ANOVA for repeated measures with fixed effects was used for each analysis.19 The Greenhouse-Geisser epsilon was used to adjust for multi-sample asphericity in the significance tests of within-subject effects.20 After tests on main effects, multiple comparisons, vertical (between treatment groups) and horizontal (between sample times), were carried out using the Dunn-Bonferroni procedure adjusted for repeated measures maintaining an experiment-wise alpha level of 0.05.19 A MANOVA was carried out utilizing a linear combination of the data from the 4 plasma proteins and using the same repeated measures design. Hotelling’s T² statistic was calculated for the treatment group, pressure devices, and sample times effect.21 Significance levels were measured at ≤0.05 throughout. Pearson product-moment correlations between plasma proteins were computed separately for patient and control groups. The significance level for each correlation coefficient was based on a 2-tailed test at the P=0.05 level.22

Results
Changes in Plasma Levels of Factor VII, TFPI, Prothrombin Fragment F1.2, and Antithrombin
There were no significant differences between the 2 groups in the baseline values of any of the proteins measured. FVIIa levels declined significantly (P<0.001) during IPC in both normal subjects and patients (Figure 1A) and with each device. The levels at 60, 120, and 180 minutes were lower compared with baseline levels in both groups. There was a significant (P<0.001) difference in FVIIa levels over time between the 2 groups; the 180 minutes levels were lower in the normal subjects (P<0.05) (18% to 24% of baseline values) compared with patients (40% to 43%) (P<0.05). There were no significant differences between the devices in either group.

Factor VII antigen levels reflect total circulating FVII in plasma. Although the ANOVA revealed a significant time effect (P=0.006), the multiple comparisons indicated no consistent pattern across time in either group for FVII antigen (Figure 1B). In normal individuals a small increase (P<0.05) was observed at 60 and 120 minutes over basal levels during IPC using 1 device (TSC) but not others. The FVII antigen levels were significantly higher in the patients compared with normal subjects (P=0.002); significant differences were found between groups during IPC with 2 devices (CSQ, TSQ).

Plasma TFPI levels rose in both normal subjects and patients (P<0.001) (Figure 2A). TFPI levels were higher (P<0.05) relative to baseline at 180 minutes in both groups with all devices and at 120 minutes in normal subjects with all devices, except TSC. The mean levels at 180 minutes ranged from 126% to 204% of basal in normal subjects and 125% to 133% in patients. TFPI levels during IPC were higher in normal subjects compared with patients (P<0.001). There were significant differences (P<0.05) between the groups at 60 minutes for CSC, 120 minutes for TSQ, and 180 minutes for FP and CSQ. No significant differences were observed between devices in normal subjects or patients.

The ANOVA for repeated measures indicated a significant change over time (P=0.002) in plasma F1.2 levels and differences between the normal subjects and patients (P<0.001) (Figure 2B). Although there was an increasing trend over time for several devices in normal subjects, the changes in F1.2 were not significant. Normal subjects had higher F1.2 levels than patients but this could not be confirmed by multiple comparisons. No significant differences were observed between devices.

Results of a multivariate analysis combining data from the 4 plasma protein measurements (FVIIa, FVII antigen, TFPI, and F1.2) reinforced the between groups differences found on the univariate analyses (not shown).

No significant differences were observed either over time or between groups in plasma antithrombin activity (not shown). FVIIa, FVII antigen, and TFPI levels were also measured in sequential samples from 3 normal subjects who...
were handled in an identical manner to the study subjects but not subjected to IPC devices. No significant changes were observed over 180 minutes.

**Relationships Between Changes in FVII, TFPI, and F1.2**

To understand the nature of the changes occurring in plasma during IPC we explored the relationships between the plasma levels of the proteins measured. The relationships were analyzed separately for the normal subjects and patients using pooled data from all the devices. No relationship was observed in either group between FVII antigen and FVIIa levels. There was an inverse relationship in both groups between plasma TFPI and FVIIa (Figure 3) (normal subjects: \( r = -0.31, P = 0.001 \); patients: \( r = -0.37, P < 0.001 \)). An inverse but weaker relationship was also observed between TFPI and FVII antigen levels in normal subjects (\( r = -0.23; P = 0.012 \)) but not patients. There was a direct relationship between F1.2 and TFPI levels that was observed in normal subjects (\( r = 0.37; P = 0.001 \)) (Figure 4) but not in patients. Of
particular interest was a direct relationship in normal subjects between plasma TFPI and F1.2 levels even when only baseline data were examined ($r=0.56, P=0.002$) (Figure 4) suggesting a stimulatory effect of thrombin generation on TFPI release into plasma.

**Discussion**

The major findings in this study are that IPC induces a rise in plasma levels of TFPI, the principal regulator of the TF pathway of blood coagulation, with a concomitant decrease in plasma FVIIa, the activated form of FVII, in both normal subjects and patients with venous disease. An inverse relationship was observed (Figure 3) between plasma TFPI levels and FVIIa levels. Although the number of normal subjects and patients studied is small, each subject was studied with each of 5 different IPC devices with a uniformly consistent trend. Circulating blood normally contains picomolar concentrations of FVIIa,\textsuperscript{16} which could trigger the initial FVIIa-TF catalyzed activation of factors X and IX when blood is exposed to extravascular tissue factor and thereby initiate blood coagulation. The observed increase in TFPI with a decrease in FVIIa suggests that IPC induces an inhibition of the earliest events in the activation of blood coagulation by the TF pathway. TFPI is synthesized primarily in the endothelial cells.\textsuperscript{22} There are at least 3 distinct pools of TFPI in vivo.\textsuperscript{7} The largest pool (~80% to 85%) remains associated with the endothelial surface.\textsuperscript{7} The plasma pool constitutes 10% and 80% of this circulating associated with lipoproteins.\textsuperscript{23,24} Approximately 3% of total TFPI is present in the platelet pool. The pool associated with the vascular endothelium is released into the plasma by heparin.\textsuperscript{12,13} The heparin-releasable pool may constitute the major pool responsible for the anticoagulant effect of TFPI.\textsuperscript{25} We postulate that the observed increase in plasma TFPI levels after IPC originates from the endothelial cells and may result from the following 2 mechanisms: by displacement from endothelial surface (as occurs after heparin infusion)\textsuperscript{12,13} and secretion from the intracellular stores. This would lead to an increase in plasma of full-length carrier-free TFPI, which is a more efficient anticoagulant than the C-terminally truncated and lipoprotein bound TFPI in plasma.\textsuperscript{26,27} Although plasma levels of free TFPI were not measured in our study, this would imply that the increase in TFPI anticoagulant activity would be higher than that reflected by increase in the total plasma TFPI levels observed in our subjects.

Our studies show a striking decline in FVIIa levels during IPC (Figure 1) without a concomitant change in FVII antigen (Figure 2). FVIIa cannot be neutralized effectively unless it is bound to TF,\textsuperscript{5–7} and the 2 plasma proteinase inhibitors that inhibit FVIIa-TF catalytic activity are TFPI \textsuperscript{5–7} and antithrombin.\textsuperscript{28–31} No changes were observed in plasma antithrombin levels in our subjects. The inverse relationship between FVIIa levels and TFPI (Figure 3) in both groups of subjects suggests a role of TFPI in the decline in FVIIa. TFPI inhibits FVIIa by a mechanism that requires FXa,\textsuperscript{5–8} and the rate-limiting step for the inactivation of FVIIa-TF appears to be the initial formation of TFPI-FXa complex.\textsuperscript{32} The small “idling” amounts of FXa that are continuously produced under basal conditions even in normal individuals\textsuperscript{6} may fulfill this requirement. It has been suggested\textsuperscript{33} that plasma TFPI levels are regulated by constant low levels of FXa activation that occurs under basal conditions.\textsuperscript{6} Our findings are in line with this hypothesis. In our studies, there was a correlation between plasma F1.2 (a product of the action of FXa on prothrombin) and TFPI levels in normal subjects (Figure 4). Indeed, a relatively strong relationship was observed even in baseline plasma samples from normal subjects ($r=0.56, P=0.002$) (Figure 4). Moreover, IPC may induce minimal
localized activation of the TF pathway (possibly via activation of monocytes or endothelial cells) leading to some additional FXa and thrombin generation and endothelial release of TFPI. Thrombin can rapidly release TFPI from endothelial sources. Although plasma F1.2 levels were not markedly elevated in our studies (Figure 2), a localized and limited thrombin generation that is rapidly controlled may not result in a discernible increase in peripheral plasma levels. The hypothesis that a low level generation of FXa and thrombin lead to coagulation inhibitory mechanisms via TFPI is supported by previous observations that infusion of low levels of thrombin leads to an anticoagulant effect. Lastly, the specific mechanisms by which IPC induces a release of TFPI or causes a decline in FVIIa remain to be defined, and it is conceivable that they are unrelated and due to mechanisms other than those postulated above.

There were differences between normal subjects and patients in some of the measurements. Plasma TFPI levels during IPC were lower ($P<0.001$) in the patients (Figure 2), suggesting that presence of underlying postthrombotic venous disease may blunt the increase in TFPI levels. This may be related to chronic endothelial dysfunction with impaired TFPI synthesis and secretion or to chronic depletion of the endothelial pool of TFPI, similar to that observed after administration of heparin. Consistent with the diminished TFPI, the FVIIa levels were significantly higher at 120 and 180 minutes in the patients, although baseline levels were comparable in the 2 groups (Figure 1). FVII antigen levels were slightly higher in patients, and this has been observed in patients with vascular diseases. Overall, the differences between patients and normal subjects, in the rise in TFPI and decline in FVIIa, may suggest a diminished antithrombotic effect of IPC in patients with severe venous disease. Lastly, our studies failed to detect major differences between the effects of different devices, possibly related to the small number of subjects. Further studies in a larger number of subjects are needed to clarify the involved mechanisms and differences between devices.

In summary, our studies demonstrate that IPC results in an increase in plasma TFPI and a decrease in FVIIa levels indicating inhibition of TF-dependent pathway. We postulate that IPC induces release of TFPI from endothelial TFPI pool. Inhibition of the TF pathway, the major physiological initiating mechanism of blood coagulation, may be an important mechanism for the antithrombotic effect of intermittent pneumatic compression.

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