# Ticlopidine and Aspirin Pretreatment Reduces Coagulation and Platelet Activation During Coronary Dilation Procedures

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*Objectives.* It is unknown whether a therapeutic combination of aspirin (ASA) and ticlopidine might effectively decrease activation of hemostasis.

*Background.* Percutaneous transluminal coronary angioplasty (PTCA), rotational atherectomy and stent implantation are procedures that fracture or ablate endothelium and plaque, a situation that activates hemostasis.

*Methods.* In 85 patients undergoing PTCA for a $77.8 \pm 1\%$ stenosis, we measured markers of coagulation and platelet activation (thrombin-antithrombin complexes [TAT], prothrombin fragment $1 + 2$ [$F_{1+2}$] serotonin and the presence of circulating activated platelets reacting with monoclonal antibodies against glycoproteins exposed on platelet membranes). Blood samples were drawn from a peripheral vein and from the coronary ostium before the procedures. Both immediately and 10 min after angioplasty, and 10 min afterward, samples were collected from a probing catheter (0.018 in. [0.46 cm]) positioned beyond the stenosis. All patients were being treated with antianginal drugs and ASA, 250 mg/day. Seventy of them had taken ticlopidine, 250 mg, twice daily for $\leq 1$ day ($\leq 24$ h) (n = 28) or for $\geq 3$ days ($\geq 72$ h) (n = 42). Heparin (150 U/kg) was administered before angioplasty. Thirty patients underwent PTCA; 15 of them were not treated with ticlopidine and 15 were given ticlopidine ($\geq 72$ h). Thirty-five patients had stent implantation, 20 rotational atherectomy.

*Results.* Before and during the procedures, there was greater thrombin generation (expressed by higher TAT and $F_{1+2}$ plasma levels) in patients not taking ticlopidine or taking it for $\leq 24$ h ($p < 0.05$). Platelet activation and plasma serotonin levels were also significantly higher in the no ticlopidine or $\leq 24$-h ticlopidine groups.

*Conclusions.* The combined use of ticlopidine, ASA and heparin effectively controls activation of coagulation in patients with stable or unstable angina undergoing coronary dilation.

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Percutaneous transluminal coronary angioplasty (PTCA) is an alternative procedure to surgical revascularization in the presence of atherosclerotic coronary artery disease (1,2). Rotational atherectomy (3) and stent implantation (4,5) further expand PTCA indications to calcified inelastic plaques and to complicated irregular lesions. PTCA produces endothelial desquamation, splitting of the atheromatous plaque, dissections and fibrous tissue rupture (6). The abrasion of the endothelium and the rupture of the plaque expose fatty plaque (which contains tissue factor) and smooth muscle to platelets, inducing activation of the hemostasis system (2,7–11). The generation of thrombin and the intracoronary deposition of activated platelets may be responsible for acute and subacute

thromboses that limit the immediate and short-term success of PTCA (1,2,7–9,12). In addition, serotonin locally released from the dense granules of the circulating activated platelets is thought to contribute to post-PTCA coronary vasoconstriction (13). Clinical studies with intracoronary injection of serotonin suggest that serotonin affects coronary artery tone (14,15), its vasoconstrictor or vasodilator effect being dependent on the presence of a normal endothelium (9,10,14,15). We (16) have previously reported that alpha-adrenergic receptor antagonists are able to counteract post-PTCA coronary vasoconstriction.

Patients undergoing angioplasty are usually treated with heparin and aspirin (ASA) (1–3). ASA (17–19) inhibits the prostaglandin G/H synthase, ultimately reducing the formation of thromboxane $A_2$, whereas heparin (20), acting as a cofactor, enhances antithrombin III activity against thrombin. Despite this antithrombotic regimen, acute and subacute thromboses remain a problem in the outcome of PTCA (4,5,8,9,12). With the advent of new devices, such as stents that fracture and the Rotablator, which completely obliterates endothelium, plaque and smooth muscle cells or sometimes the adventitia, effective and safe antithrombotic strategies are needed to avoid acute occlusion without causing bleeding complications (3–5,21).

In this study we investigated the degree of platelet and

**Abbreviations and Acronyms**

ASA = aspirin
$F_{1+2}$ = prothrombin fragment 1 + 2
ISDN = isosorbide dinitrate
$PGE_1$ = prostaglandin $E_1$
PTCA = percutaneous transluminal coronary angioplasty
TAT = thrombin-antithrombin

coagulation activation in coronary artery blood samples obtained during dilation procedures to test the hypothesis that the addition of a second antiplatelet drug such as ticlopidine (22) might further attenuate platelet activation and plasma serotonin release and consequently attenuate post-PTCA distal vasoconstriction (13).

## Methods

**Patient selection.** We enrolled in the study 85 patients (67 men, mean age $\pm$ SD 63.8 $\pm$ 10 years) with documented myocardial ischemia who were undergoing PTCA for significant stenoses of major epicardial vessels (mean $\pm$ SEM stenosis 77.8 $\pm$ 1%). Patients were included in the study after giving informed written consent. They had no major diseases other than angina pectoris. Sixty-five patients had unstable angina. Twenty of the 30 patients undergoing conventional angioplasty had stable angina and were considered to be at lower thrombosis risk; 15 of these were not treated with ticlopidine. Five of the 15 patients of the PTCA group had unstable angina and consequently were given full antianginal treatment and ticlopidine for $\geq$72 h. In these five patients with unstable angina no stents were implanted because balloon dilation gave a satisfactory result. Forty-eight patients had significant stenosis of the left anterior descending coronary artery, 12 of the left circumflex coronary artery, 23 of the right coronary artery and 2 of the left main trunk.

**Study protocol.** The protocol was approved by the Clinique Pasteur Ethical Committee and is summarized in Figure 1. All patients were pretreated with calcium channel antagonists (diltiazem, 180 to 360 mg/day), intravenous, oral or topical nitrates and ASN (250 mg/day) starting $\geq$10 days before PTCA. Patients who underwent rotational ablation or stent implantation were given a beta-adrenergic blocking agent (atenolol, 50 to 100 mg/day).

**Dilation procedure.** Neuroleptic analgesia with droperidol (2 to 10 mg intravenously) and phenoperydine (0.6 to 1 mg intravenously) was administered during the PTCA procedure according to the standard protocol of the Clinique Pasteur (16). With the patient under local anesthesia obtained with 1% xylocaine, we cannulated a radial artery with a 6F sheath (35 patients) or a femoral artery with either 6F or 8F sheaths (50 patients).

Selective coronary visualization of the stenotic vessel was obtained in two projections to provide the most accurate evaluation of the stenotic region avoiding visual vessel overlapping. The same projections were subsequently repeated soon after and 15 min after the dilation procedures to document vasoconstriction (13,16,23). The angiographic images were acquired with a Philips-DCI single-plane system at a cine rate of 25 frames/s, with a 6.5-in. (16.5 cm) image intensifier mode. Meglumine ioxaglate (64 g iodine/200 ml) was used as a nonionic contrast medium. At the beginning of the PTCA procedure a 0.014-in. (0.36 cm) guide wire was inserted far beyond the stenosis and left in place throughout the study. A 0.018-in. (0.46 cm) 300-cm long guide wire was used in Gianturco-Roubin stent implantation. A 0.009 guide wire was inserted in rotational ablation.

PTCA was performed by standard technique using a mono-rail balloon catheter system that provided the fastest catheter exchange and insertion of a probing catheter for drawing blood samples. Heparin (150 U/kg) was administered intravenously at the beginning of the procedure, to keep the clotting time >300 s (24). The activated clotting time was measured 10 and
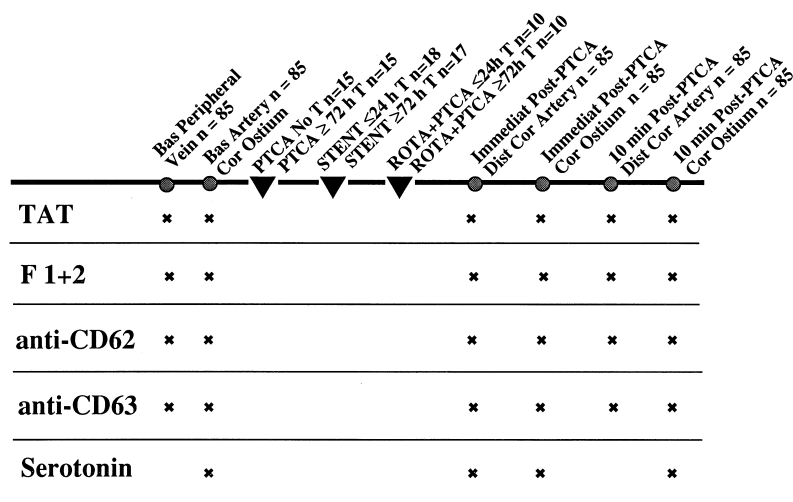
**Figure 1.** Chart of study protocol. anti-CD62 and anti-CD63 = positive platelet activation; Bas = basal; Cor = coronary; Dist = distal; F 1+2 = prothrombin fragment 1+2; Immediat = immediately; No T = no ticlopidine; PTCA = percutaneous transluminal coronary angioplasty; ROTA = Rotablator; Serotonin = plasma serotonin levels; T = ticlopidine; TAT = thrombin-antithrombin complexes.

**Table 1.** Vasoconstriction in the PTCA and Control Vessels

| Vessel | No. | Normal Vessel (mm) | Stenosis mm | Stenosis % | Procedure | Immediately Post PTCA (mm) | 15 Min Post PTCA (mm) | 15 Min Post-PTCA Elastic Recoil Vasoconstriction (%) |
|---|---|---|---|---|---|---|---|---|
| PTCA | 15 | 2.72 ± 0.2 | 0.67 ± 0.1 | 75 ± 3 | PTCA | 2.20 ± 0.2 | 1.50 ± 0.2* | −30 ± 3* |
| Distal | 15 | 2.02 ± 0.2 | | | ≥72 h T | 1.92 ± 0.2 | 1.71 ± 0.2* | −16.3 ± 2* |
| Control | 10 | 2.15 ± 0.2 | | | No ISDN | 2.10 ± 0.2 | 1.80 ± 0.2* | −16.3 ± 2* |
| | | | | | | | | |
| PTCA | 15 | 2.83 ± 0.2 | 0.62 ± 0.1 | 78 ± 2 | PTCA | 2.29 ± 0.2 | 1.63 ± 0.2* | −27 ± 2* |
| Distal | 15 | 2.08 ± 0.2 | | | No T | 2.14 ± 0.2 | 1.73 ± 0.2* | −17.4 ± 2* |
| Control | 12 | 2.13 ± 0.1 | | | No ISDN | 1.97 ± 0.1 | 1.20 ± 0.1* | −15.5 ± 2* |
| | | | | | | | | |
| PTCA | 10 | 2.76 ± 0.1 | 0.55 ± 0.1 | 79 ± 3 | Rotablator | 2.1 ± 0.1 | 1.32 ± 0.13* | −37 ± 6* |
| Distal | 10 | 1.82 ± 0.2 | | | ≥72 h T | 1.86 ± 0.6 | 1.61 ± 0.2* | −16.6 ± 3.1* |
| Control | 9 | 2.55 ± 0.3 | | | ISDN, 3 + 6 mg | 2.64 ± 1.0 | 2.27 ± 0.3* | −16.3 ± 1.7* |
| | | | | | | | | |
| PTCA | 10 | 2.57 ± 0.1 | 0.52 ± 0.5 | 79 ± 1 | Rotablator | 2.11 ± 0.1 | 1.46 ± 0.1* | −31 ± 2* |
| Distal | 10 | 1.75 ± 0.1 | | | ≤24 h T | 2.01 ± 0.1 | 1.58 ± 0.1* | −15.24 ± 3.1* |
| Control | 10 | 1.96 ± 0.1 | | | ISDN, 3 + 6 mg | 2.15 ± 0.1 | 1.75 ± 0.2* | −11.2 ± 2.1* |
| | | | | | | | | |
| PTCA | 17 | 3.20 ± 0.2 | 0.75 ± 0.1 | 77 ± 2 | Stenting | 3.02 ± 0.1 | 2.81 ± 0.1* | −7.3 ± 2* |
| Distal | 17 | 2.28 ± 0.2 | | | ≥72 h T | 2.21 ± 0.2 | 1.92 ± 0.1* | −13.9 ± 2.8* |
| Control | 10 | 2.24 ± 0.3 | | | ISDN, 3 mg | 2.34 ± 0.2 | 2.09 ± 0.3* | −7.15 ± 3.5 |
| | | | | | | | | |
| PTCA | 18 | 3.03 ± 0.2 | 0.67 ± 0.1 | 78 ± 1 | Stenting | 2.88 ± 0.2 | 2.63 ± 0.2* | −9.3 ± 2* |
| Distal | 18 | 1.92 ± 0.2 | | | ≤24 h T | 1.99 ± 0.2 | 1.73 ± 0.2* | −11.4 ± 2.7* |
| Control | 11 | 2.05 ± 0.2 | | | ISDN, 3 mg | 2.07 ± 0.2 | 1.87 ± 0.2 | −6.8 ± 4.8 |

*$p < 0.05$: % Elastic Recoil is calculated versus post-PTCA diameter, and % Vasoconstriction is calculated versus basal diameter. Data presented are mean value ± SEM. ISDN = isosorbide dinitrate; Normal Vessel = reference vessel diameter; % Elastic Recoil = percent reduction of diameter measured at the site of dilation; % Vasoconstriction = diameter reduction measured at a distal level of the dilated vessel or at a distal level of the control vessel; PTCA = percutaneous transluminal coronary angioplasty; T = ticlopidine.

60 min after heparin injection, that is, at the beginning and at the end of the procedure. Patients with unstable angina undergoing stent implantation or rotational atherectomy were treated before the procedure with intracoronary injection of 1 mg of linsidomine (Corvasal, Hoechst 1 mg vials), a nitrous oxide donor, and 3 mg of isosorbide dinitrate (ISDN). Repeated 3-mg doses of ISDN were injected during the procedure for patients treated with rotational atherectomy (up to 9 mg) (Table 1). Patients undergoing conventional PTCA were not given nitrates during the procedure (Table 1).

The number of inflations, the amount of pressure (atm) and manipulation of the Rotablator were determined by the operator. One or multiple stents (Gianturco-Roubin or Palmaz-Schatz) were implanted in vessels with a diameter >3.0 mm and were overexpanded as compared with the nominal vessel diameter to obtain a satisfactory result by covering the culprit lesion and properly reconstructing the coronary artery segment. Immediately after the last deflation of the balloon, a probing catheter (Cook or Schneider 3F) was quickly inserted through the tip of the monorail side and positioned in the dilated vessel beyond the stenosis to gently collect blood samples through the 0.018-in. lumen.

**Blood sampling.** After heparin had been injected, basal blood samples were collected first from a peripheral vein,

without a tourniquet, using a 19-gauge needle (Bas Vein). Subsequently, the guiding catheter was positioned at the coronary ostium and the guide wire was inserted into the distal coronary artery. At this time a basal blood sample was drawn from the coronary ostium. Two different precooled plastic syringes were used for each sample. Immediately after collection the blood was placed in two ice-chilled plastic tubes, one containing EDTA, imipramine, prostaglandin $E_1$ (PGE$_1$) and theophylline, used for plasma serotonin measurements (13,25), and one containing sodium citrate, used for coagulation measurements and to assess platelet activation after incubation of whole blood with monoclonal antibodies (9:1 vol/vol). After angioplasty, blood samples were obtained from the dilated coronary vessel with a 0.018-in. probing catheter positioned beyond the lesion and from the proximal coronary artery pulling back and washing the probing catheter. Blood samples were also collected 10 min after the dilation procedures. The protocol is summarized in Figure 1. All laboratory measurements were performed without knowledge of patient data.

**Measurement of platelet activation.** With flow cytometry one can detect in vivo platelet activation after the reaction of citrated whole blood with monoclonal antibodies (26–28) directed toward glycoproteins exposed on activated platelets. Two monoclonal antibodies reacting with CD62 and CD63

were employed (Immunotech, Marseilles, France). CD62 is a 140-kD component of the platelet alpha granule membrane and CD63 is a 53-kD lysosomal protein, expressed on the plasma membrane of platelets that have undergone the release reaction (26). Immediately after withdrawal, blood samples were incubated in the catheterization laboratory with FITC-labeled $F(ab)_2$ fragments of the two monoclonal antibodies for 20 min at room temperature, and after 20 min of incubation were fixed with 1% formaldehyde. A fluorescence-activated cell sorter (Facstar, Becton Dickinson) was used for analysis, which was performed within 24 h after fixation. Results were expressed as percent of fluorescence-positive platelets (27,28).

**Plasma serotonin measurements.** Blood was placed into ice-chilled plastic tubes containing EDTA (5 mmol/liter), imipramine (5 µmol/liter) $PGE_1$ (10 µmol/liter) and theophylline (1 mmol/liter) to prevent in vitro platelet activation and reuptake of serotonin by platelets (13,25). Samples were then centrifuged at 1,200 g for 20 min at 4°C to obtain platelet-poor plasma, which was subsequently filtered through a 0.20-µm pore size sterile filter to obtain platelet free plasma. Plasma was then stored at −80°C until serotonin was determined in duplicate by radioimmunoassay with commercially available kits (Serotonin I-125 RIA Test, DDV Biochemie, Marburg, Germany).

**Thrombin-antithrombin (TAT) complexes and prothrombin fragment 1+2 ($F_{1+2}$).** TAT complexes form in plasma when thrombin is neutralized by antithrombin III. $F_{1+2}$ forms in plasma when prothrombin is transformed into thrombin on the action of activated factor X. Both are considered markers of thrombin generation. TAT and $F_{1+2}$ were measured by enzyme immunoassays using commercially available, widely employed and validated kits (29) (Behring Enzygnost Diagnostic Inc.: ELISA-TAT Micro or ELISA-$F_{1+2}$ Micro, Marburg, Germany) on platelet-poor plasma obtained by centrifugation of citrated blood samples at 1,200 g for 15 min at 4°C.

**Quantitative angiographic analysis.** Catheter stems of known size and full of contrast medium were used for calibration: the 35-mm cine frames were analyzed by a geometric and densitometric quantitative analysis (QCA-ARTREK) described by Mancini et al. (30). The frames were automatically corrected for radiographic pincushion distortion. Diameters were determined at the narrowest stenotic point under basal condition, soon after PTCA and 15 min after PTCA to document the presence of vasoconstriction. The distal segment corresponds to a peripheral segment of the dilated artery and of the control nonmanipulated coronary artery, as we (16) and others (13,23) have described.

**Statistical analysis.** The results are expressed as mean value ± SEM. Two-way analysis of variance for repeated measures was performed with a commercial package (Sigma Stat Jandel Scientific). Table 1 presents both absolute and normalized (%) values. In view of the large interindividual variability of the absolute values, multiple comparisons between groups were performed with normalized data. Regression analysis was performed by the least squares technique. A p value < 0.05 was considered significant.

# Results

**Changes in coronary diameters induced by PTCA.** Successful coronary dilation was achieved in all patients. The data are shown separately for each group in Table 1. Fifteen minutes after PTCA, there was significant vasoconstriction in the dilated and control segments (16). Despite repeated intra-coronary injection of high doses of nitrates, patients undergoing rotational atherectomy showed marked vasoconstriction along the dilated and nonmanipulated coronary tree. The patients with stent implantation had minor but significant vasoconstriction at the stent level (−7.6 ± 3%, p < 0.05 vs. Stent+PTCA) (Table 1). There were no associations between the degree of vasoconstriction, serotonin levels or the degree of hemostasis activation.

**Coagulation activation.** The activated clotting time measured during the procedures was 391 ± 29 s and 397 ± 30 s, respectively, at 10 min and 60 min after heparin injection (mean heparin given 9,572 ± 261 IU).

Mean plasma levels of TAT complexes and $F_{1+2}$ (Fig. 2 and 3, respectively) were not significantly different in venous and arterial baseline samples, indicating that the presence of the catheter did not activate coagulation with the antithrombotic treatment employed. In patients not taking ticlopidine or taking it for <24 h (Fig. 2 and 3), mean baseline levels of both measurements were higher than in the comparison group of healthy persons studied to establish the laboratory reference values. During the coronary dilation procedures the degree of coagulation activation did not change significantly, with no differences found between basal venous and arterial levels of TAT and $F_{1+2}$ as compared with levels immediately after and 10 min after PTCA (Fig. 2 and 3). Significantly higher TAT levels were found in patients not treated or treated with ticlopidine for ≤24 h than in patients given ticlopidine pretreatment for ≥72 h (Fig. 2). Figure 3 also shows that $F_{1+2}$ values tended to be higher in the ≥72-h ticlopidine-treated groups, but the difference was statistically significant for only a few samples.

**Platelet activation.** A status of platelet activation (expressed as a higher proportion of platelets reacting with both monoclonal antibodies than in control samples reacting with $F[ab]_2$-FITC) was observed in patients not treated or treated with ≤24-h ticlopidine (p < 0.05 vs. ≥72-h ticlopidine). Because platelet activation was similar in groups treated or not treated with ticlopidine whatever the procedure performed, the results were pooled and are shown in Figure 4. Patients treated with ≥72-h ticlopidine had a degree of platelet activation similar to that seen in a comparison group of patients undergoing coronary angiography because of a false positive effort test and showing a normal coronary artery tree and negative findings on angina-provocative tests (Fig. 4).

**Plasma serotonin levels.** Plasma serotonin concentrations in 68 patients were measured in blood drawn from the coronary ostium and from the distal coronary artery immediately after and 10 min after the procedures. Basal coronary ostium levels and levels obtained immediately after dilation
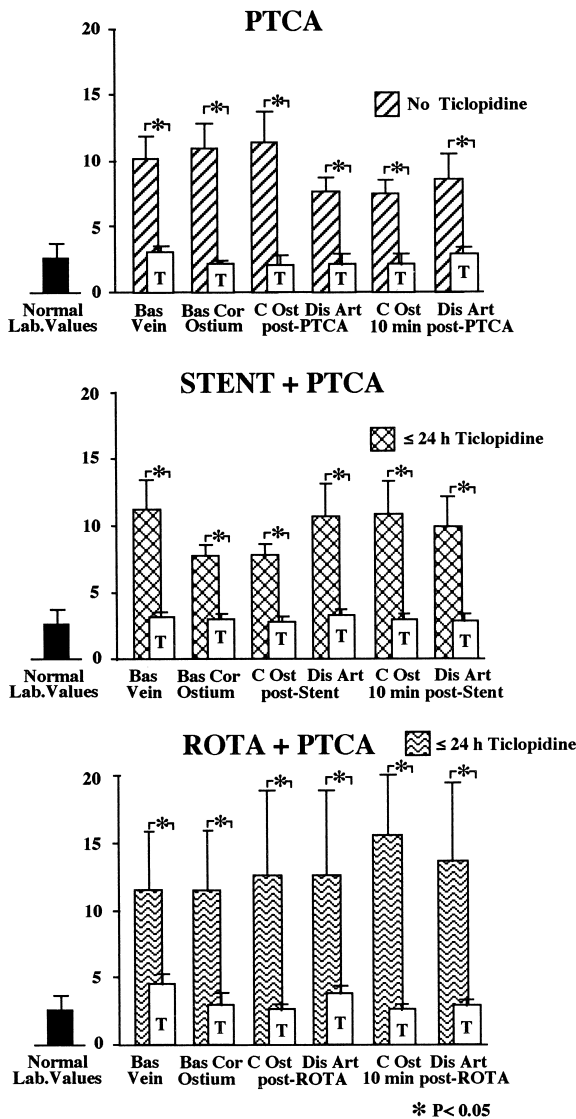
**Figure 2.** Intraprocedure thrombin generation. Bar graphs showing mean values ± SEM of thrombin-antithrombin complexes (TAT) during percutaneous transluminal coronary angioplasty (PTCA) stent implantation + PTCA and rotational ablation (ROTA) + PTCA. C Ost = coronary ostium; Dis Art = distal coronary artery measurements obtained on blood samples drawn from the coronary ostium and distal coronary artery immediately after and 10 min after PTCA; Lab. = laboratory; T = ≥72-h ticlopidine; other abbreviations as in Figure 1.



**Figure 3.** Intraprocedure thrombin generation. Bar graphs showing mean values ± SEM of prothrombin fragment 1+2 (F 1+2) during the dilation procedures. Abbreviations as in Figure 2.

procedures did not differ significantly from distal coronary artery levels. The results are reported in Figure 5. Only differences between patients treated for ≥72 h and patients who received either no ticlopidine or ticlopidine for ≤24 h were statistically significant (p < 0.05).

When serotonin levels were plotted against the degree of elastic recoil and vasoconstriction observed 15 min after the procedure, we found no significant correlation.

**Bleeding complications.** There was no bleeding severe enough to require blood transfusion or local bleeding in patients during and after the procedures.
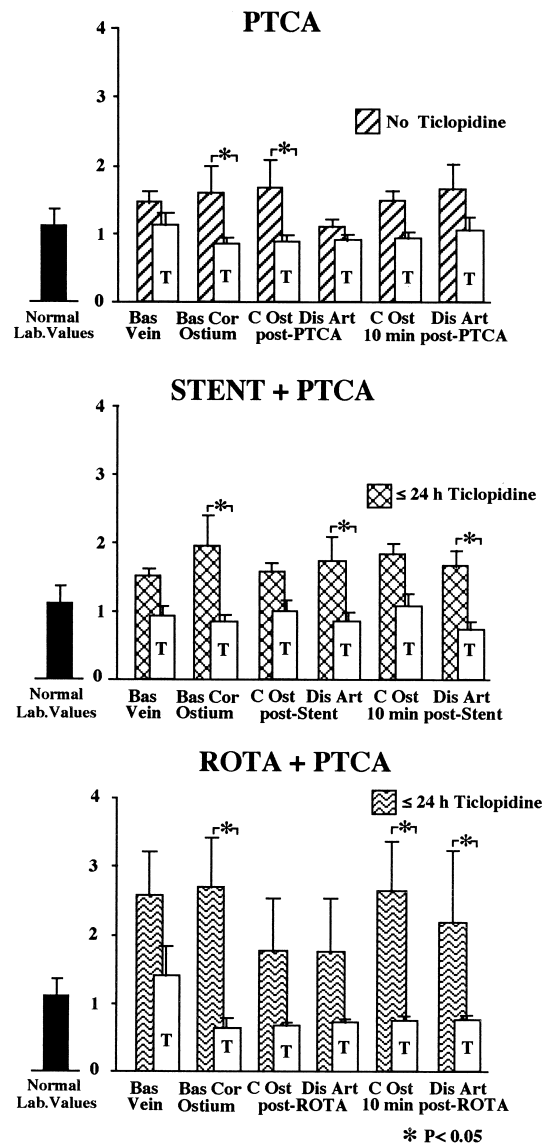
## Discussion

This study shows that combining ticlopidine (given for ≥72 h) with ASA and heparin is more effective than ASA and heparin alone or ticlopidine given for ≤24 h in decreasing the degree of activation of the hemostatic system in patients with stable or unstable angina even before the performance of coronary dilation procedures. During the procedures, the three-drug antithrombotic regimen maintained the hemostasis activation at the low state achieved before the procedure. In other studies (12,31,32), ASA and heparin alone were not able to completely counteract some activation of the coagulation cascade that leads to acute thrombotic events (1,4,5).

In this study, to evaluate the activation of hemostasis during coronary dilation procedures, blood was drawn from the distal
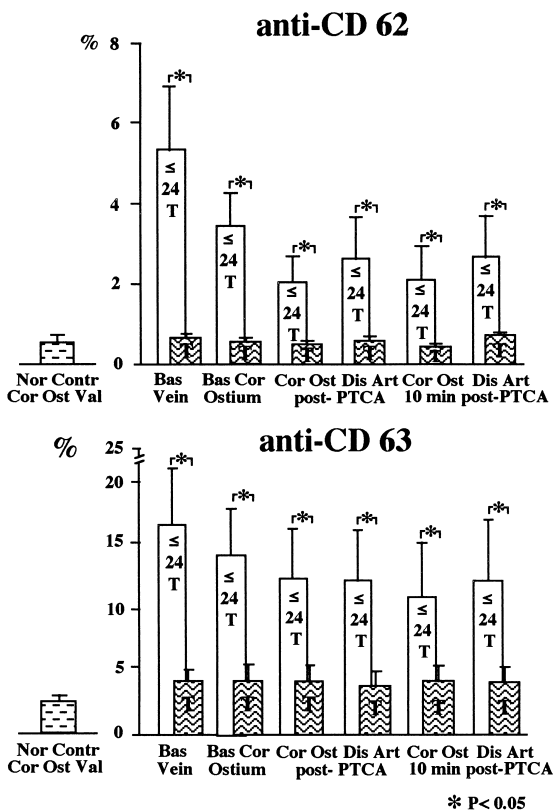
**Figure 4.** Intraprocedure in vivo platelet activation (n = 74). Bar graphs showing percent anti-CD62 positive and anti-CD63 platelet binding. No differences in platelet activation were found in groups undergoing different procedures; consequently patients have been grouped according to ≤24 h or ≥72 h ticlopidine (T) therapy. Nor Contr Cor Ost Val = normal values, normal coronary artery control values (see Platelet activation, under Results). Other abbreviations as in Figures 1 and 2.
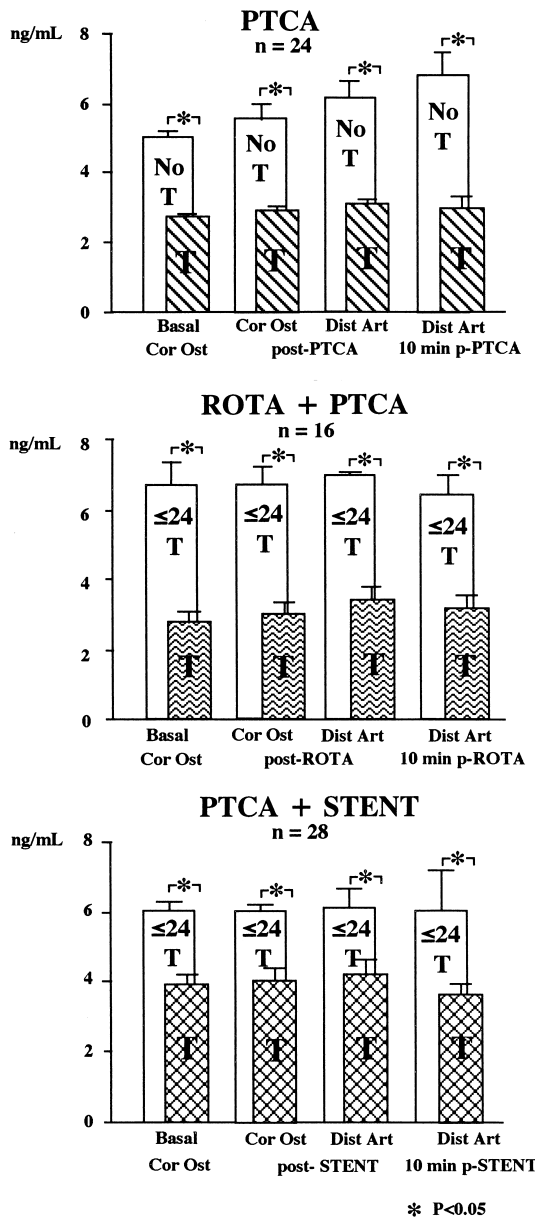


**Figure 5.** Bar graphs showing mean value ± SEM of plasma serotonin levels: p- = post; p-ROTA = postrotational ablation and stent implantation; other abbreviations as in Figures 1 and 2.

segment of the same artery rather than from the coronary sinus (13). In this way we avoided 1) mixing of blood derived from different territories, 2) the confounding effect of a potential release of serotonin synthesized and metabolized by myocardial cells (33), or 3) active endothelial/platelet reuptake of serotonin along the coronary microcirculation (34,35).

**Behavior of hemostasis measurements.** Activation of the coagulation system was explored by measuring the plasma concentrations of TAT complexes and $F_{1+2}$. Both measurements are sensitive and specific markers of thrombin generation, which is central not only to coagulation but also to activation of platelets (36). At all times during the procedures of coronary dilation, TAT complex levels were much lower in patients treated than in those not treated with ticlopidine. The same trend was seen for $F_{1+2}$, although the differences were smaller than those for TAT and at most times were not statistically significant. The reasons for the differences in behavior of the two markers of thrombin generation are not clear, but it has been previously observed (37) that in patients with ischemic heart disease TAT complexes may be more sensitive indexes than $F_{1+2}$ of the activation of coagulation

induced by thrombolytic agents. In parallel with lesser activation of the coagulation system, platelets were less activated in ticlopidine-treated patients, as shown by lower plasma serotonin levels and less exposure on the platelet membranes of the glycoproteins that indicate the occurrence of the platelet release reaction.

**Rationale for combining two antiplatelet drugs.** The rationale for combining the two antiplatelet agents ASA and ticlopidine consists in their different mechanisms of action, which should lead to more drastic suppression of platelet function. ASA permanently inactivates prostaglandin G/H synthase, resulting in the suppression of thromboxane-dependent platelet activation (19). Ticlopidine (22) seems to

be involved in three pathways of platelet activation: it inhibits adenosine diphosphate (ADP)–induced platelet activation (38), decreases the exposure of fibrinogen receptor on the platelet membrane and inhibits serotonin release from platelets (39). However, it is an important limitation in the use of this drug, especially in the course of an acute coronary event requiring PTCA, that in most persons ~72 h is required for the drug to be fully active (22,39). This is consistent with our findings that ticlopidine had to be administered for ≥72 h to decrease the degree of activation of hemostasis, whereas it was no more effective than ASA plus heparin alone when administered for ≤24 h.

**Effect of anti-ischemic treatment.** Our patients were also treated with calcium channel antagonists, which, in experimental animals, reduce platelet deposition and thrombus formation and prolong bleeding time (40,41). Nitrous vasodilators also inhibit platelet activation through complex mechanisms that might involve increased production of endogenous nitric oxide and of cyclic guanosine monophosphate (GMP) while lowering intracellular cytosolic calcium (42,43). Hence, these treatments may have contributed to the control of hemostasis activation in our patients.

**Serotonin release and vasoconstriction.** The combined antithrombotic treatment did not affect the previously observed vasoconstrictor response that occurs after PTCA (16). When the protocol included the use of the Rotablator, there was marked vasoconstriction despite repeated intracoronary injections of nitrous oxide donors. In a previous report (16) we hypothesized that the occurrence of such coronary vasoconstriction, in both the dilated and control vessels was the result of a sympathetic cardiocardiac excitatory reflex (44,45) elicited by mechanical manipulation of the vessel. Golino et al. (13) hypothesized that serotonin release from activated platelets was responsible for the coronary vasoconstriction, as they found that it was reduced by the administration of ketanserin. However, ketanserin also has an alpha$_1$-adrenergic receptor blocking activity (46). In our study coronary blood concentrations of serotonin were not appreciably increased by angioplasty, probably because of our pharmacologic treatment, and yet the magnitude of coronary vasoconstriction was unmodified. The occurrence of vasoconstriction along a nonmanipulated vessel seems to reinforce the hypothesis that a sympathetic reflex rather than a released circulating substance is predominantly involved in this phenomenon.

**Clinical implications.** The addition of ticlopidine for ≥72 h to ASA and heparin markedly reduces the activation of coagulation and platelets. These findings are consistent with experimental data suggesting that the inhibition of platelets with combinations of ASA and ticlopidine may attenuate factor Xa/Va activity, and, thus, thrombin generation (22,47). Recently, a clinical trial (48) performed in a large series of patients demonstrated that this combination of drugs significantly reduces acute and subacute occlusions and at the same time reduces bleeding complications more than do oral anticoagulant agents (4,5).

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