Pharmacokinetics of Tissue-Type Plasminogen Activator During Acute Myocardial Infarction in Men
Effect of a Prostacyclin Analogue

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Background. Coronary reocclusion complicates the thrombolytic therapy of acute myocardial infarction despite the routine use of aspirin. This is consistent with experimental studies demonstrating that multiple agonists, in addition to thromboxane A2, mediate the platelet activation underlying reocclusion. Consequently, a more potent antiplatelet therapy with a broader spectrum of activity than aspirin may be required in this setting. Prostacyclin and its more stable analogue, iloprost, inhibit platelet aggregation to all known agonists and exert an additional effect over aspirin alone. Experiments in animal models have demonstrated, however, that iloprost increases the clearance of tissue-type plasminogen activator (t-PA) and impairs thrombolysis in vivo. This study examines whether a similar interaction occurs in humans.

Methods and Results. Twelve patients with acute myocardial infarction received t-PA intravenously, 60 mg in the first hour and a maintenance infusion of 13.3 mg/hr for 3 hours. Patients were assigned in a double-blind fashion to iloprost (2 ng/kg/min) or placebo following the initial 90 minutes of the maintenance infusion of t-PA. Iloprost decreased mean arterial blood pressure (−10 ± 2.9 mm Hg, p < 0.05) but did not alter heart rate. Steady-state plasma iloprost concentration was 591 ± 64 pmol/l. At this concentration, iloprost markedly inhibited platelet aggregation in vitro, particularly in the presence of aspirin. Steady-state clearance of t-PA was unchanged by iloprost (454 ± 65 versus 443 ± 136 ml/min in controls, p = NS). Furthermore, neither elimination kinetics nor plasma protein binding of t-PA was altered by iloprost.

Conclusions. At plasma levels that exert a potent antiplatelet effect, iloprost did not alter the pharmacokinetics of t-PA in men. Prostacyclin analogues may prove useful as an adjunct to plasminogen activators, particularly in patients at high risk for thrombotic reocclusion. (Circulation 1992;85:526–532)

Thrombolytic therapy reduces mortality1,2 and improves left ventricular function in patients with acute myocardial infarction.3 The clinical response is modest, however, despite rates of reperfusion as high as 75%.4 Two factors may account for this.

First, continuing thrombosis triggered by platelet activation may result in reocclusion, which complicates 15–25% of cases.5–7 Biochemical evidence of platelet activation8,9 and thrombin formation10 has been demonstrated in patients receiving tissue-type plasminogen activator (t-PA) and streptokinase. There is also evidence that antiplatelet therapy improves the response to coronary thrombolysis.11 Second, additional myocardial injury may occur after reperfusion. The mechanism of this reperfusion injury is uncertain but appears to involve leukocyte activation and infiltration of the myocardium.12

Studies in experimental models demonstrate that potent inhibition of platelets alone can prevent reocclusion.13–17 These data further suggest that platelet activation during coronary thrombolysis involves mul-
tiple agonists, including thrombin,13 thromboxane (TX) A2,13-16 and serotonin.16 Consequently, inhibition of TXA2 alone (as achieved with aspirin) may be inadequate to prevent reocclusion.6,15 A broader spectrum of antiplatelet activity can be achieved with prostacyclin (PGI2) and its more stable analogues. These compounds abolish platelet activation and aggregation to all known agonists18 and prevent reocclusion in animal models of coronary thrombosis, whereas inhibition of TXA2 alone is ineffective.15,19,20 Prostacyclin and its analogues also protect against reperfusion injury,21-23 possibly by inhibiting neutrophil migration and activation.24 A potential concern with this approach has been the finding in animal models that a prostacyclin analogue, iloprost, impairs thrombolysis, possibly by increasing the clearance of t-PA.19,25 The clinical relevance of these reports is uncertain, because iloprost was administered in doses orders of magnitude higher than are required in humans. In this study, we examined the pharmacokinetics of t-PA and iloprost when coadministered in patients with acute myocardial infarction. These studies demonstrate that at plasma concentrations that exert potent antiplatelet effects, iloprost does not interfere with the clearance of t-PA.

Methods

Materials

5’[(E)-(1S,5S,6R,7R)-7-Hydroxy-6’-[(E)-(3S,4RS)-3-hydroxy-4-methyl-1-octen-6-inyl)bicyclo[3.3.0]octan-3-ylidenyl]-pentanoidic acid (iloprost) was provided by Dr. W. Dole, Berlex Inc., Wayne, N.J. (15S)-Hydroxy-11,9-(epoxymethano)-prostadienoic acid (U46619) was a gift from Dr. R. Gorman, Upjohn Co., Kalamazoo, Mich. t-PA was purchased from Genentech Inc., South San Francisco, Calif., and was largely of the single-chain type. ADP was obtained from Sigma Chemical Co., St. Louis, Mo., collagen from Bio/Data Corp., Hatboro, Pa., and the other materials as indicated.

Study Protocol

The study group consisted of patients admitted with chest pain of cardiac origin who exhibited ECG evidence of an acute myocardial infarction. All patients gave written informed consent. Patients were randomized to receive iloprost or placebo in a double-blind manner. t-PA (100 mg) was administered intravenously over 4 hours. A total of 60 mg was infused over the first hour with the administration of a bolus of 0.1 mg/kg (from a minimum of 6.0 mg to a maximum of 10.0 mg). The remaining 40 mg was infused over the subsequent 3 hours (maintenance infusion). Midway through the maintenance infusion, intravenous iloprost or placebo was commenced and continued over the subsequent 48 hours. If the patient tolerated an initial infusion of 1.0 ng/kg/min of iloprost, the rate was increased to 2.0 ng/kg/min after 10 minutes. The infusion rate was decreased in decrements of 0.5 ng/kg/min if intolerable side effects occurred. Two hours before discontinuation of iloprost, the infusion rate was decreased by 50%, and by a further 50% 1 hour before its discontinuation.

Sampling Protocol

Blood samples were taken via an indwelling catheter from a forearm vein contralateral to the site of t-PA infusion. Samples for antigenic t-PA determination were collected into sodium citrate (3.8%, 9:1 vol/vol) and centrifuged immediately, and the plasma was stored at -70°C. Samples of venous blood were also acidified with citrated acetic acid (pH 3.9), and the plasma was separated immediately for subsequent fibrin autography.

Blood samples for determination of t-PA antigen were collected at the following time points: 1) after the change in t-PA infusion to the maintenance infusion rate at 0, 1, 2.5, 5, 10, 15, 20, 30, 45, 60, 70, and 80 minutes; 2) after the additional infusion of iloprost or placebo at 30, 60, 70, 80, and 90 minutes; and 3) after cessation of t-PA (but during the continued infusion of iloprost or placebo) at 1, 2.5, 5, 10, 15, 20, 30, 45, 60, and 90 minutes. Acidified samples were collected at intervals during the maintenance infusion of t-PA before and after the addition of the iloprost or placebo infusion.

Biochemical Analysis

Plasma t-PA was determined by an enzyme-linked immunosorbent assay (ELISA) (American Diagnostica Inc., Greenwich, Conn.) as described previously.26 In addition to determining total (or antigenic) t-PA concentration by ELISA, we performed fibrin autography27 to analyze the fractions of t-PA that were free or bound to its inhibitors. Samples were collected in acidified citrate, added to sampling buffer, and applied to a 7.5% polyacrylamide gel with a stacking gel of 4%. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis,28 the gel was washed in 0.25% Triton X-100, overlaid on a fibrin plate, and incubated for 16 hours.27 Plasminogen activators were identified as areas of lysis on the fibrin gel detected both by protein staining and by dark-ground illumination. Iloprost concentrations were determined by a modification of the method of Krause et al.29 Briefly, iloprost was extracted from plasma along with an internal standard, deuterated iloprost, with an affinity column containing an anti-iloprost antibody coupled to Sepharose as the stationary phase. The extracted iloprost was derivatized with pentafluorobenzyl bromide and bis(trimethylsilyl)-trifluoracetamide. The derivatized iloprost was chromatographed on a CP-Sil-5 CB fused capillary column and then detected using negative ion-chemical ionization mass spectrometry with selected ion monitoring at m/z 503 and 508 for iloprost and its deuterated analogue, respectively.

Pharmacokinetic Analysis

The concentrations of t-PA at steady state (60, 70, and 80 minutes during the maintenance infusion and 70, 80, and 90 minutes after the initiation of iloprost
Platelet Aggregation

Blood for 15 minutes, platelet-poor plasma was prepared by centrifuging the remaining blood at 900g for 10 minutes. Agonists were added in volumes of 10% or less to 500-μl aliquots of platelet-rich plasma, and the threshold concentration, the lowest concentration to induce maximum aggregation, was identified.

Statistical Analysis

Results are expressed as mean±SEM. Each patient acted as his own control (before versus during iloprost), allowing the analysis of data by paired Student's t test.

Results

Twelve male patients were studied, of whom seven received iloprost and five received placebo (Table 1). Steady-state concentrations of t-PA were not attained during the maintenance infusion in one patient per group. In one of these patients, marked variability in plasma t-PA occurred after an episode of ventricular tachycardia. Both patients were excluded from further analysis of t-PA pharmacokinetics before the blinded randomization code was broken. Medications before t-PA administration included aspirin (n=10), nitroglycerin (n=10), morphine (n=8), lidocaine (n=6), cimetidine (n=2), diltiazem (n=1), and nifedipine (n=1).

Effects of Iloprost Infusion

All patients tolerated a final infusion rate of iloprost (2 ng/kg/min) over the period of sample collection. Infusion of both iloprost and placebo was associated with headache and pyrexia. Nausea was reported by six of the seven iloprost-treated patients, but by none of the control group (Table 1) (p<0.005). The nausea was present at more than 12 hours of iloprost infusion in four of these six patients. Nausea was unrelated to the site of myocardial infarction. Iloprost was continued for 48 hours, and the dose was decreased by half before its discontinuation to avoid rebound hemodynamic or platelet effects. In all cases, iloprost was withdrawn without any problems. Steady-state plasma concentrations of iloprost were 591±64 pmol/l and fell to 302±44 pmol/l after the dose was halved. Thus, the plasma clearance of iloprost was 797±65 ml/min.

Both groups demonstrated a similar fall in hemoglobin (14.9±0.7 to 12.9±0.3 mg/dl by day 2 in controls; 15.3±0.6 to 13.7±0.8 mg/dl in the iloprost-treated group). One patient on iloprost had blood-streaked vomitus, with little change in hematocrit (17.6 to 16.2 mg/dl). Peak plasma creatine phosphokinase–MB was 204±52 units/ml at 10.8±3.0 hours in the iloprost group and 245±124 units/ml at 11.8±0.2 hours in the controls. None of these differences were significant. Five patients underwent cardiac catheterization; of these, three of four patients receiving iloprost and the one control patient had a patent culprit vessel at cardiac catheterization. All patients had resting radionuclide or contrast ventriculography before discharge. The ejection fractions were 47±6% and 51±14% (p=NS) in the iloprost- and placebo-treated groups, respectively.

Platelet Aggregation Studies

Platelet aggregation in response to ADP (2.5–10 μmol/l), collagen (5–190 μg/ml), and U46619 (0.13–2.0 μmol/l) was determined by light transmission. Platelet-rich plasma was prepared by centrifuging citrated whole blood (3.8% sodium citrate, 9:1 vol/vol) at 150g for 15 minutes, and platelet-poor plasma was prepared by centrifuging the remaining blood at 900g for 10 minutes. Agonists were added in volumes of 10% or less to 500-μl aliquots of platelet-rich plasma, and the threshold concentration, the lowest concentration to induce maximum aggregation, was identified.

Hemodynamic Response

After administration of iloprost, mean arterial blood pressure fell from 97±3 to 87±4 mm Hg (p<0.02), whereas heart rate was unaltered (86±6 versus 83±6 beats per minute). In the control group, none of these hemodynamic indexes were altered; mean arterial blood pressure was 101±10 mm Hg before and 102±6 mm Hg after the vehicle, and heart rate was 81±7 versus 85±7 beats per minute, respectively.

Pharmacokinetics of t-PA

Figure 1 demonstrates the plasma levels of t-PA throughout the infusion protocol for controls and patients receiving iloprost. After reduction to the maintenance infusion rate at 1 hour, plasma t-PA concentra-
tion fell to a steady-state level. Iloprost or vehicle was infused beginning 90 minutes into the 3-hour maintenance infusion and continued for 48 hours. Before initiation of the placebo or iloprost, steady-state t-PA clearance was 573±80 ml/min in the control group (n=4) and 563±83 ml/min (n=6) for the group who later received iloprost (Table 2). t-PA clearance was unchanged during the infusion of iloprost (454±65 ml/min, p=NS) or placebo (443±136 ml/min, p=NS).

After discontinuation of the t-PA infusion at 4 hours, t-PA concentration declined in a biexponential manner both in the control and in the iloprost-treated patients. In one patient per group, the elimination of t-PA exhibited a single prolonged terminal phase with half-lives of 277 and 444 minutes, respectively. In the control group, the initial α half-life was 4.0±0.8 minutes (accounting for the majority of the plasma drug elimination) and the terminal β half-life was 70±15 minutes. In the iloprost-treated group, the corresponding values were 4.8±2.0 and 55±9 minutes, respectively, and were not significantly different from placebo-treated controls. Furthermore, in this group, the α half-life when t-PA was discontinued was similar to that obtained on switching to the maintenance infusion before any iloprost was given (Table 2). After reduction to the maintenance infusion rate, after the first hour of t-PA, the α half-life was 4.1±1.5 minutes in the iloprost-treated patients (p=NS). The corresponding value in the control group was 5.3±1.7 minutes.

To characterize the immunogenic material detected by the ELISA and address the possibility that iloprost altered the disposition of t-PA in plasma, fibrin autography was performed on selected samples (Figure 2). As previously demonstrated, plasma t-PA was largely in the free, active form during the infusion period.31,32 A minor amount of the t-PA was complexed to plasminogen activator inhibitor (PAI)-1, α2-antiplasmin, C1-inhibitor, and α2-macroglobulin. Iloprost had no effect on the type or extent of complex formation. Note that the complex with α2-antiplasmin is less obvious over time. This is consistent with the reduction in α2-antiplasmin that occurs in pa-
patients treated with t-PA and reflects systemic plasminogen activation.

Effect of Iloprost on Platelet Aggregation

To determine the effect of the concentration of iloprost achieved in this study on platelet function, we examined platelet aggregation in the presence and absence of iloprost 550 pmol/l. Blood was obtained from five healthy volunteers before and 1 hour after the administration of aspirin (325 mg p.o.). Platelet aggregation to threshold concentrations of ADP, U46619, and collagen was markedly suppressed (Figure 3). Platelet aggregation was more sensitive to iloprost after aspirin administration. Thus, aspirin reduced the IC50 of iloprost for inhibition of platelet aggregation by ADP (2.3 x 10^-10 versus 2.1 x 10^-7 mol/l, p<0.05) and collagen (7.3 x 10^-10 versus 2.5 x 10^-8 mol/l, p<0.05), both of which are dependent on TXA2 for their effect. In contrast, aspirin did not alter the IC50 (5.2 x 10^-10 versus 9.4 x 10^-10 mol/l, p=NS) for inhibition of aggregation induced by U46619, a TXA2 mimic.

Discussion

Although aspirin decreases mortality in patients receiving streptokinase,11 reocclusion still occurs in up to 25% of patients after coronary thrombolysis.5–7 This is most likely to occur in patients undergoing percutaneous coronary angioplasty34 or those who suffer recurrent ischemia.35 Potent antiplatelet therapy has been shown to prevent reocclusion in experimental models of coronary thrombolysis.13–17 In contrast, aspirin and more specific TXA2 inhibitors fail to prevent reocclusion when used alone.13,15,16 These findings suggest that the platelet activation underlying reocclusion is mediated by many agonists. Prostacyclin and its stable analogues, such as iloprost, have a broad spectrum of antiplatelet activity,18 preventing aggregation to all known agonists. They are also potent coronary vasodilators and have been reported to prevent reperfusion injury.21–23 PGI2 and iloprost have proved effective in preventing reocclusion in canine models of coronary thrombolysis. Furthermore, Vaughan and colleagues36 have demonstrated that PGE2, which activates PGI2 receptors,37 increases the rate of clot dissolution by t-PA in the rabbit. Thus, PGI2 and iloprost may prove to be useful and novel adjuncts to thrombolytic therapy.

Despite these beneficial effects, two recent studies in a canine model of coronary thrombosis have demonstrated that iloprost increased t-PA clearance and inhibited lysis in vivo.19,25 Thus, in both studies, iloprost decreased plasma t-PA levels and delayed or prevented reperfusion. A similar mechanism was postulated to explain a lower but not statistically significant difference in coronary patency in a pilot study of iloprost (2 ng/kg/min) in humans.30 In a number of species, the clearance of t-PA is largely hepatic and is dependent on liver blood flow.39,40 Prostacyclin and its analogues are potent vasodilators and increase liver blood flow in humans41,42; consequently, they may increase t-PA clearance. In the animal studies, however, the dose of iloprost required to prevent reocclusion and inhibit platelet aggregation ex vivo was 100–200 ng/kg/min.19,20,25 At this dose, there was a marked fall in arterial blood pressure. In contrast, platelet aggregation in humans is inhibited at a dose that is two orders of magnitude lower.43 Thus, it may be possible to achieve a plasma concentration of iloprost in humans that inhibits platelets without producing a marked hemodynamic effect or change in t-PA clearance.

There has been no previous study of the pharmacokinetics of t-PA that used the presently approved dosing regimen or over such a prolonged infusion. Nevertheless, the pharmacokinetic indexes of t-PA derived from our patient group agree well with those reported for the predominantly one-chain form of the plasminogen activator given as a short infusion or as a bolus. Garabedian et al44 reported a clearance for t-PA of 451–636 ml/min at infusion rates up to 9.5 µg/kg/min in patients with acute myocardial infarction. A similar clearance (550 ml/min) has been reported in patients with peripheral vascular disease receiving 0.25 mg/kg t-PA over 10 minutes.45 Although in our study group, there was a slight reduction in clearance over time (443±136 versus 573±80 ml/min), this difference did not achieve statistical significance. The elimination of t-PA was best fitted by a biexponential function, with a rapid initial phase (t1/2α, 4.0–4.8 minutes) accounting for 90% of the total clearance. This agrees well with the 3.6–4.6 minutes reported by Garabedian et al.44

Iloprost was administered beginning midway through the infusion of low-dose t-PA and continued for 48 hours, the period of highest risk for reocclusion. This protocol allowed us to examine the pharmacokinetics of the plasminogen activator on and off iloprost in the same individual. The dose of iloprost was selected to provide a plasma concentration that would suppress platelet aggregation while avoiding deleterious hemodynamic effects.46 At the plasma concentration
achieved, iloprost exerts a potent antiplatelet effect, particularly in platelets first exposed to aspirin. Ex vivo platelet aggregation was not studied in our patient population because of the confounding effects of other medications, including t-PA, that may produce artifacts during sampling. In previous studies, however, this dose of iloprost inhibited ex vivo platelet aggregation in healthy volunteers,\textsuperscript{47} in patients with stable angina,\textsuperscript{48} and in acute myocardial infarction.\textsuperscript{49} There is also evidence of in vivo antiplatelet effects in humans at equivalent doses of prostacyclin. Thus, the plasma concentration of $\beta$-thromboglobulin was reduced and platelet survival increased in patients with peripheral vascular disease.\textsuperscript{50} These effects were evident even in the absence of aspirin. The clearance of iloprost in our patients with acute myocardial infarction was $797\pm65$ ml/min. This is about half of that reported in healthy young volunteers.\textsuperscript{51}

The clearance of t-PA was unaltered by iloprost, the plasma concentration curves being superimposable. Similarly, iloprost did not alter the initial half-life of the plasminogen activator. Because the antibody used in the ELISA does not distinguish free and bound t-PA, we also examined the effect of iloprost on the disposition of t-PA in plasma. t-PA forms SDS-stable complexes with a number of plasma proteins, including PAI-1, $\alpha_2$-antiplasmin, C1-inhibitor, and $\alpha_2$-macroglobulin.\textsuperscript{32} The complexes formed are irreversible at neutral pH and are inactive. They can be identified by fibrin autography because the complex is dissociated in the gel and active t-PA released. These studies demonstrated that the majority of the t-PA infused in humans is in the free, active form and that iloprost has no effect on its disposition in plasma. Thus, the data derived from the immunological assay of t-PA are not confounded by alterations in protein binding.

The present study demonstrates that a platelet-inhibitory concentration of iloprost can be achieved in vivo without inducing a marked hemodynamic effect or altering the clearance of t-PA. This latter finding will also apply to other doses of t-PA, because its clearance follows first-order kinetics and is unrelated to dose in the therapeutic range.\textsuperscript{45} One disadvantage with iloprost was the frequent occurrence of nausea, particularly early during the infusion of t-PA. Prostacyclin or other analogues may be less likely to cause this side effect.\textsuperscript{52} However, nausea was more frequent than reported in normal volunteers and may reflect the decreased clearance of iloprost in patients with acute myocardial infarction. Whether these compounds will prove effective in the setting of coronary thrombosis is as yet unclear. A small pilot study reported no significant effect of iloprost on the response to t-PA in humans.\textsuperscript{38} The effect of such therapies, however, may not be evident from small clinical trials. ISIS-2, which demonstrated the benefit of combining aspirin with streptokinase, involved more than 17,000 patients.\textsuperscript{11} Furthermore, iloprost and similar compounds may be most effective if targeted toward patients at risk of reoclusion. Thus, the additional antiplatelet effect may be useful in patients undergoing early coronary angioplasty\textsuperscript{44} or who have recurrent ischemia despite aspirin and heparin administration.\textsuperscript{35}

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References

13. Fitzgerald DJ, Fitzgerald GA: The role of thrombin and thromboxane A\textsubscript{2} in vascular reocclusion following coronary thrombolysis with tissue type plasminogen activator. Proc Natl Acad Sci USA 1988;85:7755–7759
14. Shebuski RJ, Smith JM, Storer BA, Granett JR, Bugelski PJ: Influence of selective endoperoxide/thromboxane A\textsubscript{2} receptor antagonism with sulotroban on lysis time and reocclusion rate.
after tissue plasminogen activator-induced coronary thrombolysis in the dog. J Pharmacol Exp Ther 1988;246:790–796
43. Yardumian DA, Mackie IJ, Brennan EC, Bull H, Machin SJ: Platelet function studies during and after infusion of ZK 36374, a stable prostacyclin analogue, to healthy volunteers. Prostaglandins 1986;31:26–26

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