Journal of the American College of Cardiology
© 2003 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 42, No. 8, 2003
ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1097(03)01059-3

Effect of Activated Protein C on Plasma Plasminogen Activator Inhibitor Activity in Patients With Acute Myocardial Infarction Treated With Alteplase

Comparison With Unfractionated Heparin

Tomohiro Sakamoto, MD,* Hisao Ogawa, MD,* Keiji Takazoe, MD,* Michihiro Yoshimura, MD,* Hideki Shimomura, MD,‡ Yasushi Moriyama, MD,‡ Hidekazu Arai, MD,‡ Kenji Okajima, MD†
Kumamoto and Kasuga, Japan

OBJECTIVES We examined whether activated protein C (APC) is an effective conjunctive therapy to thrombolysis in patients with ST-segment–elevated acute myocardial infarction (AMI).

BACKGROUND Activated protein C possesses both systemic anticoagulant and anti-inflammatory properties. It has been also shown to enhance fibrinolysis by inhibiting plasminogen activator inhibitor (PAI) activity in vitro.

METHODS After successful thrombolysis with alteplase, study patients were assigned to receive one of the two conjunctive therapies for 48 h intravenously: human plasma-derived APC at 0.06 mg/kg per day (APC group, n = 9) or unfractionated heparin at 100 to 400 U/kg per day, adjusted to maintain an activated partial thromboplastin time at 1.5 to 2 times of the control level (heparin group, n = 10).

RESULTS Adverse events, including reocclusion of the recanalized infarct-related coronary artery and major or minor hemorrhagic complications, occurred more frequently in the heparin group (4 of 10 cases) than in the APC group (none of 9 cases) ($p = 0.033$). In the heparin group, plasma PAI activity (IU/ml, median value [range]) was increased continuously from 8 to 24 h after thrombolysis and peaked at 24 h (30.9 [11.3 to 38.5]); on the other hand, it was not increased in the APC group at 24 h after thrombolysis (11.3 [0.0 to 31.0], $p < 0.01$ vs. heparin group).

CONCLUSIONS Administration of APC suppressed increasing of plasma PAI activity observed after thrombolysis in patients with AMI. The effect of APC could be more eligible, compared with heparin, as a conjunctive regimen to thrombolysis in AMI patients. (J Am Coll Cardiol 2003;42:1389–94) © 2003 by the American College of Cardiology Foundation

Protein C is one of the most important physiologic anticoagulant components (1,2). After activation by thrombin–thrombomodulin complexes on the membrane surface of platelets and endothelial cells, protein C is converted to activated protein C (APC), which inactivates factors Va and VIIIa (3,4). These properties of APC are thought to be eligible for a therapeutic strategy against thrombotic disorders. In fact, APC has favorable effects on a murine model of ischemic stroke (5) and a rat model of mesenteric arteriolar thrombosis (6). Activated protein C has also been shown to inactivate type 1 plasminogen activator inhibitor (PAI-1), thereby promoting fibrinolysis (7,8). When released from activated platelets, PAI-1 is thought to be involved in the formation of microthrombus by inhibiting clot lysis (9), and that may cause reocclusion of coronary arteries after thrombolytic therapy in patients with acute myocardial infraction (AMI).

From the Departments of *Cardiovascular Medicine and †Laboratory Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; and the ‡Division of Cardiology, Fukuoka Tokushukai Hospital, Kasuga, Japan.

Manuscript received October 6, 2002; revised manuscript received June 18, 2003, accepted June 24, 2003.

We have previously demonstrated that APC is more effective than heparin in preventing arterial reocclusion after thrombolysis in a canine model of coronary artery thrombosis (10). We have also reported that the endogenous APC level is elevated in patients with AMI, and the levels were higher in cases with unsuccessful thrombolysis, suggesting that APC generation might be accelerated in response to the increased thrombin generation in those cases (11). From these observations, we hypothesized that therapeutic administration of exogenous APC might be useful as a conjunctive therapy to enhance the effects of thrombolysis in patients with AMI.

To examine this hypothesis, we investigated the effect of APC conjunctively administered after thrombolysis with recombinant tissue-type plasminogen activator (t-PA) in patients with AMI. The effects were compared with those of conventional conjunctive therapy using unfractionated heparin in a randomized fashion.

METHODS

The schematic summarization of the study protocol of the present study is shown in Figure 1.

Abbreviations and Acronyms

AMI	= acute myocardial infarction
anti-PC MAb	= anti-human protein C monoclonal antibody
APC	= activated protein C
aPTT	= activated partial thromboplastin time
BSA	= bovine serum albumin
CAG	= coronary angiography
ECG	= electrocardiogram/electrocardiographic
PAI-1	= type 1 plasminogen activator inhibitor
TBS	= tris buffered saline
TNF	= tumor necrosis factor
t-PA	= tissue-type plasminogen activator

Patients. We studied 19 consecutive patients with first ST-segment–elevated AMI successfully treated with recombinant t-PA. Patients were admitted to our institutions within 6 h after symptom onset. All had ischemic symptoms lasting ≥30 min and met the following electrocardiographic (ECG) criteria: ST-segment elevation ≥0.1 mV in two or more limb leads or ST-segment elevation ≥0.2 mV in two or more contiguous precordial leads. Written, informed consent was obtained from each patient and his or her family. The study protocol was in agreement with the guidelines of the ethics committee at our institutions.

Thrombolysis. All patients underwent emergent coronary angiography (CAG) immediately after they were diagnosed as having AMI. Patients with cardiogenic shock and a contraindication to thrombolytic therapy (active peptic ulceration, previous intracerebral hemorrhage, surgical operation, significant trauma, or head injury within the last four weeks) were excluded from this study, and instead of thrombolysis, coronary angioplasty was selected for reperfusion therapy. All patients received 81 mg oral aspirin and an intravenous bolus administration of 100 U/kg unfractionated heparin (heparin sodium, Shimizu Pharmaceutical Co., Ltd., Shizuoka, Japan) before CAG. After confirming the total occlusion of an infarct-related coronary artery, corresponding to the ST-segment–elevated leads on the ECG, the patients underwent thrombolytic therapy with the recombinant t-PA called alteplase (GRTPA, Tanabe Seiyaku Co., Ltd., Osaka, Japan). Alteplase was administered intravenously at 0.5 to 0.75 mg/kg. Ten percent of the total dose was administered as an intravenous bolus, followed by drip infusion of the rest over 60 min. The maximum total dose is recommended not to exceed 41.4 mg (24 million IU). Infusion was stopped when Thrombolysis In Myocardial Infarction trial flow grade 3, confirmed by repeated CAG with continuous 12-lead ECG monitoring, was obtained.

Conjunctive therapy. Patients were randomly assigned to receive one of the following two intravenous conjunctive therapies during 48 h after thrombolysis: unfractionated heparin, 100 to 400 U/kg per day, adjusted to maintain an activated partial thromboplastin time (aPTT) at 1.5 to 2 times the control level (heparin group, n = 10), or human plasma-derived APC, 0.06 mg/kg per day (APC group, n = 9). The randomized conjunctive therapy was begun immediately after the completion of thrombolysis. Table 1 shows the baseline patient characteristics of the two conjunctive treatment groups.

Activated protein C. Human plasma-derived APC (CTC-111) was kindly provided by the Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan). The manufacturing process of APC is briefly described as follows. Protein C was purified from freshly prepared citrated human plasma by immunoaffinity chromatography using the anti-protein C monoclonal antibody column. After activation of protein C by human thrombin, APC was purified to homogeneity, essentially as described by Taylor et al. (12). Purified APC displayed a single peak on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified preparation of APC was pasteurized by heating at 65°C for 96 h in a dry state. No significant change was observed in the crossed immunoelectrophoresis, circular dichroic spectra, ultraviolet absorption spectrum, and fluorescence spectrum after heat treatment.

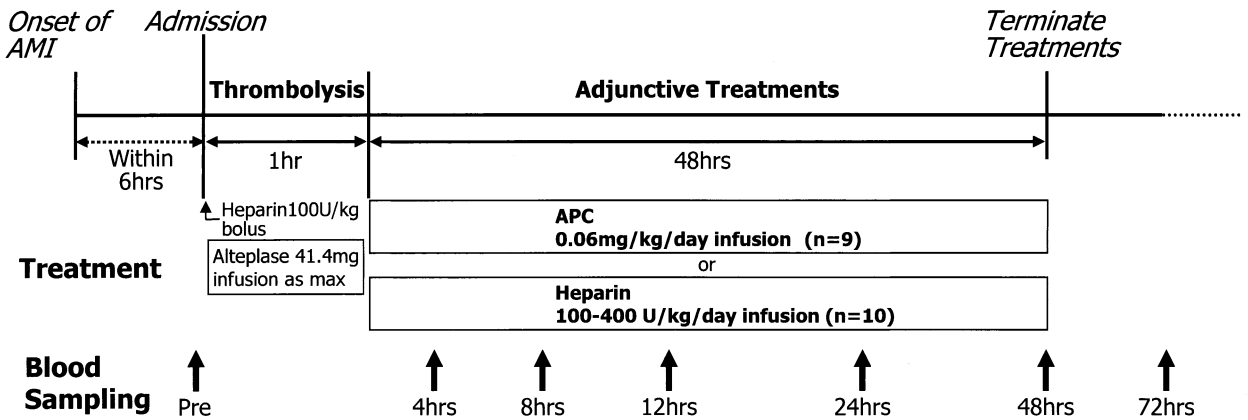

Figure 1. Schematic summarization of the study protocol. All patients were treated with a bolus injection of 100 U/kg heparin followed by a drip infusion of recombinant tissue-type plasminogen activator (alteplase). The patients were then randomly assigned to receive heparin or activated protein C intravenously for 48 h. AMI = acute myocardial infarction.

Table 1. Baseline Patient Characteristics of the Two Conjunctive Treatment Groups

	APC Group (n = 9)	Heparin Group (n = 10)
Age (yrs)	65 (38–73)	60 (49–68)
Female gender	1 (11%)	1 (10%)
Hypertension	6 (67%)	7 (70%)
Diabetes	2 (22%)	2 (20%)
BMI (kg/m^2)	24.9 (22.2–27.5)	24.8 (17.1–31.2)
Smokers	6 (67%)	7 (70%)
Total cholesterol (mg/dl)	200 (116–264)	193 (130–257)
Peak CK (U/l)	2,967 (1,072–5,637)	3,303 (1,320–4,550)
Peak CK-MB (U/l)	261 (64–421)	260 (144–400)
Reocclusion of infarct-related coronary arteries	0	1 (10%)
Bleeding complications	0	3 (30%)

Continuous data are shown as the median value (range), and the other data as the number (%) of patients.
APC = activated protein C; BMI = body mass index; CK = creatine kinase.

Blood sampling. All patients were treated in the intensive care unit during conjunctive treatments (48 h). Any evidences of adverse events, including reocclusion of the recanalized coronary arteries and intra- and/or extracorporeal bleeding, were carefully monitored. Venous blood was collected to detect peak serum creatine kinase (CK) and CK-MB levels, and following coagulation and fibrinolytic parameters; plasma levels of t-PA antigen, PAI activity, APC antigen and aPTT. Blood sampling was performed on hospital admission (pretreatment) and 4, 8, 12, 24, 48, and 72 h after the start of conjunctive treatments. At the time of sampling, the initial 3 ml blood was discarded, and the subsequent 2 ml venous blood was collected in a sequential manner into an evacuated tube containing 0.3 mol/l benzamidine for measurement of APC, and 3 ml venous blood was collected into an evacuated tube containing 0.5 ml sodium citrate (0.13 mol/l, pH 7.5) for measurement of t-PA antigen, PAI activity, and aPTT. All blood samples were centrifuged at 3,000 rpm for 10 min at 4°C, and divided plasma was stored at −80°C until analyzed.

Measurements of t-PA, PAI, APC, and aPTT. Plasma t-PA antigen levels were measured by commercially available enzyme-linked immunosorbent assay kits produced by Diagnostica Stago, Inc. (Francoville, France) (13). Intra- and interassay coefficients of variation in this assay were 2.4% and 4.7%, respectively. The control value for t-PA antigen in our laboratory (n = 35) was 5.8 ± 1.7 ng/ml (mean ± SD).

Activity levels of PAI were also measured by commercial chromogenic single-point poly-D-lysine–stimulated assay kits produced by Biopool Inc. (Umeå, Sweden) (14). Intra- and interassay coefficients of variation in this assay were 9.4% and 11.4%, respectively. The control value for PAI activity in our laboratory (n = 35) was 5.4 ± 4.1 IU/ml (mean ± SD).

Plasma APC levels were measured by enzyme capture assay with slight modifications of the method previously reported by Gruber and Griffin (15). An enzyme capture assay for APC was performed as follows: polystyrene balls were dipped in anti-human protein C monoclonal antibody (anti-PC MAb; JTC-4) (16) in phosphate-buffered saline (20 mg/ml) and incubated at 4°C overnight. Immobilization was terminated by rinsing the balls three times, followed by coating them with 1% bovine serum albumin (BSA)/phosphate-buffered saline at 4°C for two days. Plasma samples were diluted at 1:160 with 0.5% BSA/tris buffered saline (TBS). Standard APC, which was purified by affinity purification from human plasma using immobilized anti-PC MAb, was kindly provided by the Chemo-Sero-Therapeutic Research Institute. The APC standards were prepared at concentrations of 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 μg/ml, with protein C-deficient pooled normal human plasma diluted at 1:160 with 0.5% BSA/TBS. Each sample and the APC standards (400 μl) were incubated in the anti-PC MAb-fixed balls. After incubation at 37°C for 2 h, the balls were washed twice with TBS and then incubated with 400 μl of 0.05 mol/l tris-HCl buffer (pH 8.5) containing a fluorogenic peptide substrate (Peptide Institute Inc., Osaka, Japan) at 4°C overnight (17). The enzyme–substrate reaction was terminated by adding 1 ml of 0.01 mol/l acetic acid, and the fluorescence intensity was measured (excitation 380 nm, emission 460 nm). Intra- and interassay coefficients of variation in this assay were 8.1% and 3.5%, respectively. The control value for APC in our laboratory (n= 23) was 1.3 ± 1.4 ng/ml (mean ± SD).

The aPTT was determined with an automated clot timer and commercially available reagents.

Statistical analyses. Because the values obtained from this study were not normally distributed, all data are shown as median values [ranges] instead of mean values ± SD, unless otherwise noted. Because of the same considerations, nonparametric tests were used as statistical analyses. The clinical characteristics between the APC- and heparin-treated groups were compared using the Mann-Whitney *U* test for continuous data, and the chi-square test for frequency data. The time course of plasma t-PA, PAI, and APC levels and aPTT in each conjunctive treatment group was analyzed by a Friedman test followed by a multiple Wilcoxon signed rank test if H_0 was rejected. More specifically, probability levels were adjusted according to the following formula: $p_k = 1 - (1 - p_o)^k$ (i.e., the Dunn-Sidak method; p_k = adjusted p values; p_o = original p values; k = number

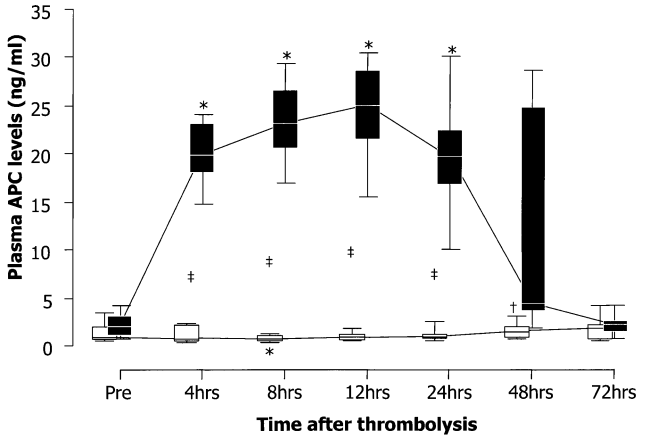

Figure 2. Serial changes in plasma levels of activated protein C (APC) between the two conjunctive treatment groups. During infusion of APC, the plasma levels were maintained at 5 to 10 times its control level. *$p < 0.05$ vs. pre in both treatment groups. †$p < 0.05$ and ‡$p < 0.01$ between the two groups at each sampling point. The **center lines** in the columns show the median value, and the **box plots** show the ranges between the 25th and 75th percentile values. The **bars attached to the columns** show the ranges between the 10th and 90th percentile values. **Solid columns** = APC group; **open columns** = heparin group.

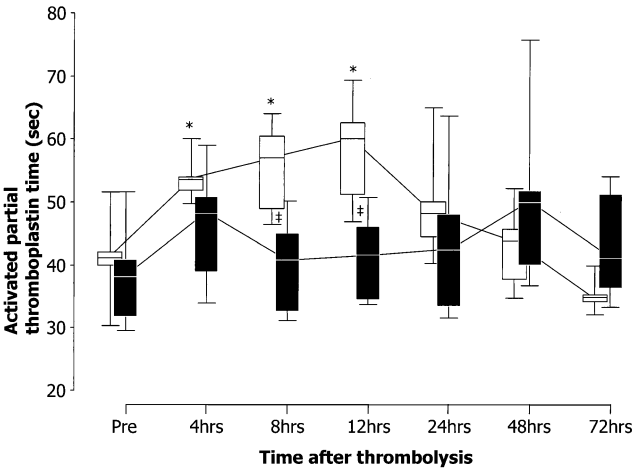

Figure 3. Serial changes in activated partial thromboplastin time (aPTT) between the two conjunctive treatment groups. The aPTT was significantly prolonged in the heparin group, though not in the activated protein C (APC) group. *$p < 0.05$ vs. pre in the heparin treatment group. ‡$p < 0.01$ between the two groups at each sampling point. The **center lines** in the columns show the median value, and the **box plots** show the ranges between the 25th and 75th percentile values. The **bars attached to the columns** show the ranges between the 10th and 90th percentile values. **Solid columns** = APC group; **open columns** = heparin group.

of tests made). Those parameters between the two conjunctive treatment groups at a specific sampling point were analyzed by the Mann-Whitney *U* test. Probability levels <0.05 were considered significant.

RESULTS

Patient characteristics and clinical course. There were no significant differences in the baseline patient characteristics and clinical courses between the two conjunctive treatment groups (Table 1). Reocclusion of the recanalized infarct-related coronary arteries was observed in one patient in the heparin group. The patient complained of chest pain at about 8 h after the start of heparin, and the ECG revealed ST-segment re-elevation in the same leads, as recorded on admission. Rescue coronary angioplasty was performed for the recanalization of the reoccluded coronary artery. On the other hand, none of the patients in the APC group had reocclusion in their recanalized coronary arteries. There were no adverse side effects, including hemorrhagic complications in the APC group, although there were two cases of minor bleeding (one with hematoma at the puncture site and macroscopic hematuria; the other with macroscopic hematuria alone), and one case of major bleeding required surgical repair (postperitoneal hematoma) in the heparin group. In this way, total adverse events occurred more frequently in the heparin group than in the APC group ($p = 0.033$).

Hemostatic parameters. Plasma APC levels (ng/ml) were significantly increased in the APC group during drip infusion (4 to 24 h, $p < 0.05$ vs. control), and the peak level reached more than 10 times the control level (2.0 [0.7 to 4.3] to 24.9 [12.9 to 31.5]) at 12 h after the start of conjunctive therapy. On the other hand, plasma APC levels were significantly decreased at 8 h (0.9 [0.5 to 4.0] to 0.7 [0.2 to 1.5], $p < 0.05$), as compared with the pretreatment level in the heparin group (Fig. 2).

In the heparin group, aPTT was significantly prolonged at 8 h and 12 h, as compared with the same sampling points in the APC group. An aPTT in the heparin group was prolonged 4 to 12 h, as compared with the pretreatment

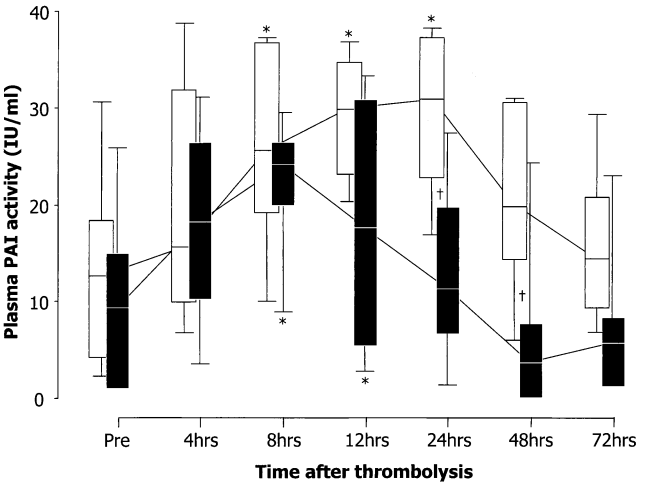

Figure 4. Serial changes in plasma levels of plasminogen activator inhibitor (PAI) activity between the two conjunctive treatment groups. There is a significant difference in the time course of PAI activity between the two groups. Activity of PAI was increased similarly until 8 h in both groups. Then the level kept increasing until 24 h in the heparin group, although it was decreasing in the APC group. *$p < 0.05$ vs. pre in both treatment groups. †$p < 0.05$ between the two groups at each sampling point. The **center lines in the columns** show the median value, and **the box plots** show the ranges between the 25th and 75th percentile values. The **bars attached to the columns** show the ranges between the 10th and 90th percentile values. **Solid columns** = APC (activated protein C) group; **open columns** = heparin group.

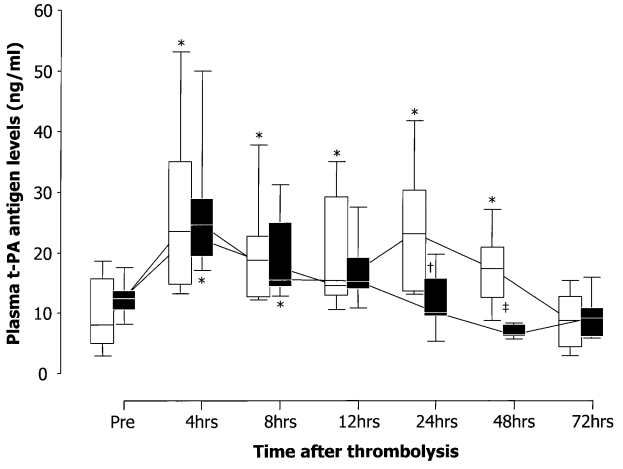

Figure 5. Serial changes in plasma levels of t-PA antigen. The level was higher at 24 and 48 h in the heparin group. *$p < 0.05$ vs. pre in both treatment groups. †$p < 0.05$ and ‡$p < 0.01$ between the two groups at each sampling point. The **center lines in the columns** show the median value, and the **box plots** show the ranges between the 25th and 75th percentile values. The **bars attached to the columns** show the ranges between the 10th and 90th percentile values. **Solid columns** = APC (activated protein C) group; **open columns** = heparin group.

level, whereas no significant changes were observed in the APC group (Fig. 3).

Plasma PAI activity (IU/ml) was increased in both groups on admission (12.7 [1.0 to 30.8] in the heparin group; 9.3 [0.0 to 30.5] in the APC group), as compared with healthy subjects. In the heparin group, plasma PAI activity was increased continuously from 8 to 24 h after thrombolysis and peaked at 24 h (30.9 [11.3 to 38.5], $p < 0.05$ vs. hospital admission value); on the other hand, it was increased only at 8 and 12 h after thrombolysis (24.1 [3.0 to 31.0] and 17.6 [1.2 to 34.4], $p < 0.05$ vs. admission) in the APC group. Plasma PAI activity was significantly suppressed in the APC group compared with the heparin group at 24 and 48 h after thrombolysis (Fig. 4).

Plasma t-PA antigen levels (ng/ml) were significantly elevated until 8 h in the APC group and until 24 h in the heparin group. The t-PA antigen levels were significantly higher in the heparin group than in the APC group at 24 and 48 h (Fig. 5).

Changes in plasma PAI activity at each of six sampling points from the pretreatment levels had an obvious relationship with those of plasma APC levels, although the relationship was not so strong. In other words, the more the plasma APC level increased, the more the plasma PAI activity decreased (Fig. 6).

DISCUSSION

In this study, for the first time, we showed that administration of APC could be a useful conjunctive therapy after thrombolysis with recombinant t-PA in patients with AMI. The effects of APC after thrombolysis, at least during first several hours, might be the overlap effects of both heparin and APC, because bolus heparin was administered to all the study patients.

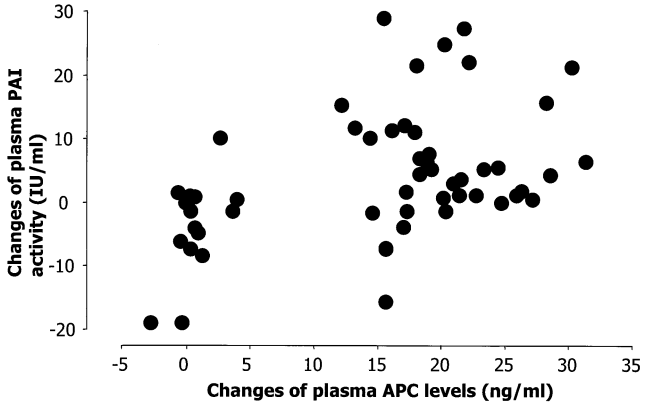

Figure 6. Correlations between changes in plasma PAI activity from the pretreatment levels and those of plasma activated protein C (APC) levels at each of six sampling points after thrombolysis in the APC group.

Anticoagulant effects of APC. Reocclusion of the recanalized coronary artery in the early phase of AMI occurs 10% to 20% of patients, and that causes deterioration of the efficacy of reperfusion therapy (18,19). Protein C is one of most important physiologic antithrombotic components (1,2). After activation by thrombin–thrombomodulin complexes on the endothelial cells, APC inactivates factors Va and VIIIa in the presence of protein S and phospholipids, which are the main constituents of the plasma membrane of platelets and endothelial cells (3,4). This means that APC acts as an anticoagulant at just the sites of thrombus generation, and it might be more effective for conjunctive therapy if administered after thrombolysis. In a canine model of coronary artery thrombosis, APC has superiority over heparin to suppress thrombotic reocclusion after recanalization with recombinant t-PA, without any hemorrhagic complications (10). The effects of APC were also confirmed in human AMI cases from the results of this study.

Profibrinolytic effects of APC. Type 1 plasminogen activator inhibitor is an important controller of net fibrinolytic activity, and plasma PAI-1 levels are increased in most AMI cases (20–22). However, in AMI cases with spontaneously recanalized infarct-related coronary arteries at emergent CAG on admission, plasma PAI activity was not increased (21). In a rabbit carotid artery thrombosis model, Fujii et al. (22) reported that plasma PAI-1 activity was increased after t-PA–induced recanalization of completely occluded vessels. On the other hand, we have reported that PAI activity is increased 4 h after thrombolytic therapy with alteplase and remains high during the first 24 h, though not in the cases with angioplasty (23). In this way, it is important to inhibit PAI-1 elevation in patients with AMI, particularly after thrombolysis. Activated protein C is known to be a unique anticoagulant because it enhances clot lysis by inhibiting PAI-1 activity in endothelial cell cultures and in clot lysis assay (7,8). Similarly, elevation of PAI activity was significantly suppressed by APC in the present study. Furthermore, it is reported that APC inhibited the production of tumor necrosis factor-α (TNF-α), which is known to

stimulate PAI-1 liberation from endothelial cells (24) and adipocytes (25), by inhibiting activation of transcriptional factors such as activator protein-1 and nuclear factor-κB (26). Thus, in addition to the direct inhibition effect on PAI-1, APC may suppress increasing of PAI-1 through an inflammatory cytokine (TNF-α) in patients with AMI. The profibrinolytic effects of APC could be contributed to less potential risk of thrombus formation as compared with heparin.

Adverse clinical events of conjunctive treatments. In this study, bleeding complications were observed only in the heparin group. However, because of a small number of study patients, it is not possible to derive meaningful insights from the events. As a matter of fact, aPTT was not so prolonged, although those events occurred during heparin infusion. Furthermore, the dose of APC used in this study was drawn upon from another study on the treatment of coagulopathy of disseminated intravascular coagulation (27). Further dose-finding trials would be needed to settle the least effective dose of APC for AMI patients to minimize possible bleeding side effects.

Conclusions. We have demonstrated that APC administered conjunctively with recombinant t-PA is a safe and effective regimen in patients with AMI. Because of several properties other than anticoagulant, including PAI-1 inhibition observed in the present study, APC could be more promising as a conjunctive therapy, as compared with conventional anticoagulants such as heparin. A large-scale, randomized trial with long-term follow-up is necessary to confirm the possible superiority of APC over other anticoagulants in patients with AMI.

Reprint requests and correspondence: Dr. Tomohiro Sakamoto, Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan. E-mail: tom@kumamoto-u.ac.jp.

REFERENCES

1. Esmon CT. Protein-C: biochemistry, physiology, and clinical implications. Blood 1983;62:1155–8.
2. Esmon CT. The regulation of natural anticoagulant pathways. Science 1987;235:1348–52.
3. Walker FJ, Sexton PW, Esmon CT. The inhibition of blood coagulation by activated protein C through the selective inactivation of activated factor V. Biochim Biophys Acta 1979;571:333–42.
4. Fulcher CA, Gardiner JE, Griffin JH, et al. Proteolytic inactivation of human factor VIII procoagulant protein by activated human protein C and its analogy with factor V. Blood 1984;63:486–9.
5. Shibata M, Kumar SR, Amar A, et al. Anti-inflammatory, antithrombotic, and neuroprotective effects of activated protein C in a murine model of focal ischemic stroke. Circulation 2001;103:1799–805.
6. Hashimoto M, Yamashita T, Oiwa K, Watanabe S, Giddings JC, Yamamoto J. Enhancement of endogenous plasminogen activator-induced thrombolysis by argatroban and APC and its control by TAFI, measured in an arterial thrombolysis model in vivo using rat mesenteric arterioles. Thromb Haemost 2002;87:110–3.
7. van Hinsbergh VW, Bertina RM, van Wijngaarden A, van Tilburg NH, Emeis JJ, Havertake F. Activated protein C decreases plasminogen activator-inhibitor activity in endothelial cell-conditioned medium. Blood 1985;65:444–51.
8. Sakata Y, Curriden S, Lawrence D, Griffin JH, Loskutoff DJ. Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity. Proc Natl Acad Sci USA 1985;82:1121–5.
9. Stringer HA, van Swieten P, Heijnen HF, Sixma JJ, Pannekoek H. Plasminogen activator inhibitor-1 released from activated platelets plays a key role in thrombolysis resistance: studies with thrombi generated in the Chandler loop. Arterioscler Thromb 1994;14:1452–8.
10. Sakamoto T, Ogawa H, Yasue H, et al. Prevention of arterial reocclusion after thrombolysis with activated protein C: comparison with heparin in a canine model of coronary artery thrombosis. Circulation 1994;90:427–32.
11. Takazoe K, Ogawa H, Yasue H, et al. Association of plasma levels of activated protein C with recanalization of the infarct-related coronary artery after thrombolytic therapy in acute myocardial infarction. Thromb Res 1999;95:37–47.
12. Taylor FB Jr., Chang A, Esmon CT, D'Angelo A, Vigano-D'Angelo S, Blick KE. Protein C prevents the coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon. J Clin Invest 1987;79:918–25.
13. Amiral J, Plassart V, Grosley M, Mimilla F, Contant G, Guyader AM. Measurement of tPA and tPA–PAI-1 complexes by ELISA, using monoclonal antibodies: clinical relevance. Thromb Res 1988;8: 99–113.
14. Eriksson E, Ranby M, Guyzander E, Risberg B. Determination of plasminogen activator inhibitor in plasma using t-PA and a chromogenic single-point poly-D-lysine stimulated assay. Thromb Res 1988; 50:91–101.
15. Gruber A, Griffin JH. Direct detection of activated protein C in blood from human subjects. Blood 1992;79:2340–8.
16. Wakabayashi K, Sakata Y, Aoki N. Conformation-specific monoclonal antibodies to the calcium-induced structure of protein C. J Biol Chem 1986;261:11097–105.
17. Ohno Y, Kato H, Morita T, et al. A new fluorogenic peptide substrate for vitamin K-dependent blood coagulation factor, bovine protein C. J Biochem (Tokyo) 1981;90:1387–95.
18. Ellis SG, Topol EJ, George BS, et al. Recurrent ischemia without warning: analysis of risk factors for in-hospital ischemic events following successful thrombolysis with intravenous tissue plasminogen activator. Circulation 1989;80:1159–65.
19. Topol EJ, Califf RM, George BS, et al. A randomized trial of immediate versus delayed elective angioplasty after intravenous tissue plasminogen activator in acute myocardial infarction. N Engl J Med 1987;317:581–8.
20. Lucore CL, Sobel BE. Interactions of tissue-type plasminogen activator with plasma inhibitors and their pharmacologic implications. Circulation 1988;77:660–9.
21. Sakamoto T, Yasue H, Ogawa H, Misumi I, Masuda T. Association of patency of the infarct-related coronary artery with plasma levels of plasminogen activator inhibitor activity in acute myocardial infarction. Am J Cardiol 1992;70:271–6.
22. Fujii S, Abendschein DR, Sobel BE. Augmentation of plasminogen activator inhibitor type 1 activity in plasma by thrombosis and by thrombolysis. J Am Coll Cardiol 1991;18:1547–54.
23. Hirashima O, Ogawa H, Oshima S, et al. Serial changes of plasma plasminogen activator inhibitor activity in acute myocardial infarction: difference between thrombolytic therapy and direct coronary angioplasty. Am Heart J 1995;130:933–9.
24. van Hinsbergh VW, Kooistra T, van den Berg EA, Princen HM, Fiers W, Emeis JJ. Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo. Blood 1988;72:1467–73.
25. Sakamoto T, Woodcock-Mitchell J, Marutsuka K, Mitchell JJ, Sobel BE, Fujii S. TNF-alpha and insulin, alone and synergistically, induce plasminogen activator inhibitor-1 expression in adipocytes. Am J Physiol 1999;276:C1391–7.
26. Yuksel M, Okajima K, Uchiba M, Horiuchi S, Okabe H. Activated protein C inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production by inhibiting activation of both nuclear factor-kappa-B and activator protein-1 in human monocytes. Thromb Haemost 2002;88:267–73.
27. Aoki N, Matsuda T, Saito H, et al. A comparative double-blind randomized trial of activated protein C and unfractionated heparin in the treatment of disseminated intravascular coagulation. Int J Hematol 2002;75:540–7.