Acute Myocardial Infarction

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

Comparison With Unfractionated Heparin

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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 infarction (AMI).

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.
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 cardiacogenic 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.
Blood sampling. All patients were treated in the intensive care unit during conjunctive treatments (48 h). Any evidence 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 to measure urine and plasma levels of t-PA, PAI, APC, 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 mol/l sodium citrate (0.13 mol/l, pH 7.5) for measurement of t-PA antigen, PAI activity, and aPTT. 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, non-parametric 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 H₀ was rejected. More specifically, probability levels were adjusted according to the following formula: pk = 1 − (1 − p₀)k (i.e., the Dunn-Sidak method; pk = adjusted p values; p₀ = original p values; k = number of comparisons).
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 level (Fig. 3). 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 group; open columns = heparin group.

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.

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.
Effect of APC after thrombolysis, at least during thrombolysis with recombinant t-PA in patients with AMI.

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.
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.

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