Protein C in Acute Stroke

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The plasma concentrations of protein C, an anticoagulant protein, and fibrinopeptide A were measured in 37 patients with acute hemispheric stroke and in age-matched controls with nonvascular neurologic diseases. In 11 stroke patients who died within 15 days after the onset (nonsurvivors) protein C antigen concentration on admission was lower than in the control group (p<0.005), with a mean value of 63% of the concentrations found in the 26 survivors (p<0.001). The difference in protein C concentrations was not associated with different prothrombin time ratios and serum albumin concentration in survivors and nonsurvivors of stroke and was independent of the size of the cerebral lesion. Increased fibrinopeptide A concentration on admission was found in all stroke patients (p<0.001), but it was higher in nonsurvivors than in survivors (p<0.01), suggesting that lower protein C concentrations in nonsurvivors might be due to increased thrombin-dependent protein C activation. In survivors, protein C concentration was slightly but significantly higher than in controls (p<0.05) and was unchanged 2 months after stroke, a time when fibrinopeptide A concentrations had returned to normal. These results show that protein C is involved in the hemostatic derangement caused by stroke and provide a rationale for clinical trials evaluating the therapeutic supplementation with protein C of patients with acute ischemic stroke. (Stroke 1988;19:579–583)

Protein C is a vitamin K-dependent zymogen of a plasma serine protease that, upon activation in vivo by thrombin complexed to endothelial thrombomodulin, potently inhibits blood coagulation through selectively inactivating factors Va and VIIIA in cooperation with protein S, another vitamin K-dependent protein.1 Activated protein C is also involved in the regulation of the fibrinolytic system by neutralizing the activity of plasminogen activator inhibitor(s)2-5 and possibly by causing release of tissue plasminogen activator.6-8

The clinical relevance of protein C in the regulation of hemostasis has become evident by the observation of recurrent thrombotic episodes in patients with congenital deficiency. Homozygotes experience extensive thrombotic disease soon after birth (purpura fulminans neonatalis) and eventually die in infancy unless they are continuously treated with plasma or prothrombin complex concentrates. Heterozygous protein C deficiency is also clearly associated with thrombotic manifestations, albeit less severe than in homozygous protein C deficiency. (For review see Clouse and Comp.)7 Although venous thromboembolism is the most frequent clinical manifestation in these patients, arterial occlusions, either isolated or in association with venous thrombosis, have been described in young protein C-deficient patients.8 Reduced plasma concentrations of protein C are also found in disseminated intravascular coagulation, in acute respiratory distress syndrome, and in the postoperative period.7 In these conditions, the low concentrations of protein C may be secondary to increased activation and turnover and, at the same time, may contribute to the development of thrombosis.

Subjects and Methods

Thirty-seven consecutive patients hospitalized within 48 hours of their first ischemic or hemorrhagic hemispheric stroke were enrolled in the study. Their mean ± SD age was 68 ± 10 years. All patients had their diagnosis confirmed by CT scan performed on admission (Day 1). A cerebral hemorrhage was diagnosed if a homogeneously hyperdense area was found. Hemorrhagic lesions were subdivided into three classes according to their size: small, lesion of up to 5 mm diameter in no more than two adjacent slices; medium,
lesion of size intermediate between small and large; and large, lesion of ≥10 mm diameter in at least five adjacent slices. In the absence of hyperdense areas, ischemic lesions were diagnosed according to their size: not visualized or small, no lesion or lesion with a maximum diameter of 5 mm visible in no more than two adjacent slices; medium, lesion of size intermediate between small and large; and large, lesion involving at least the vascular territory of the anterior, middle, or posterior cerebral artery.

Therapy aimed at maintenance of adequate blood pressure and prevention of cerebral edema was given to all patients.

CT scan was repeated on Day 5 in the 30 patients still alive; three patients (all with ischemic stroke) had evolution of the cerebral lesion from small to medium or large as defined above. With the exception of the seven patients who died before undergoing a second CT scan, the size of the lesion was determined on the basis of the lesion shown on Day 5. All patients had a follow-up of at least 60 days; those alive at the end of this period were considered survivors (n = 26). Eleven patients died during follow-up (nonsurvivors), seven by Day 5 and four between Days 6 and 15.

The control group consisted of 37 age-matched patients (mean ± SD age 65 ± 12 years) with nonvascular neurologic diseases (Alzheimer’s disease or Parkinson’s disease, amyotrophic lateral sclerosis, peripheral neuropathy, lumbar disk disease). Thirty controls had CT scan ruling out the presence of focal cerebral lesions. No control had apparent cerebrovascular disease.

No stroke patient or control received heparin or oral anticoagulant treatment before or during hospitalization.

Blood samples were obtained from stroke patients and controls on Day 1 and from 17 survivors on Days 5, 15, and 60. Venous blood was collected from an antecubital vein of the nonparalyzed arm by clean venipuncture using a 19-gauge butterfly needle. After discarding the first 3 ml, blood was drawn in a plastic syringe containing the anticoagulant supplied by the manufacturer of the FPA assay kit (see below). Blood for prothrombin time and protein C determinations was then collected in 0.13 M trisodium citrate, 0.1 M e-aminocaproic acid (1:9 vol:vol). Platelet-poor plasma was obtained by centrifuging blood at 4,800g for 15 minutes. After determination of the prothrombin time, the remaining platelet-poor plasma was deep-frozen and kept at −70°C until tested. Pooled normal plasma, snap-frozen and stored at −70°C, was made from platelet-poor plasma of 20 healthy blood donors ranging in age from 18 to 65 years, and arbitrarily assigned values of 100% for protein C concentration and of 1.0 for the prothrombin time ratio.

Concentration of protein C antigen in plasma was measured by electroimmunoassay using a specific rabbit antiserum. The anticoagulant and amidolytic activities of activated protein C were measured in a one-stage Xa clotting assay and in a chromogenic assay using S-2238 (Kabi, Stockholm, Sweden), respectively, after isolation of protein C from plasma with a calcium-dependent monoclonal antibody and activation with immobilized thrombin-thrombomodulin complex. FPA concentration was measured by radioimmunoassay (Mallinckrodt, St. Louis, Missouri) using the FPA standard provided with the kit, after blood collection and processing of the samples according to the instructions of the manufacturer. Prothrombin time was determined using human brain thromboplastin supplied by Dr. Poller (Manchester Comparative Reagent), and results are expressed as ratios of patient to control clotting time. Serum albumin concentration was evaluated by densitometric scanning after cellulose acetate electrophoresis.

Unless specified, geometric means and 95% confidence limits were calculated by logarithmic transformation of individual values. The significance of the difference between means was tested with one- or two-way analysis of variance and Student’s t test for unpaired or paired data.

Results

Mean protein C antigen concentrations in plasma were not different on admission in the 37 stroke patients and the controls (Table 1). Protein C concentrations were not significantly different in patients with ischemic and hemorrhagic stroke (Table 1). However, when stroke patients were subdivided according to survival, nonsurvivors had significantly lower protein C antigen concentrations than either survivors (p < 0.001) or controls (p < 0.005) (Table 1).

Impaired liver synthesis is one of the possible mechanisms responsible for reduced plasma protein C concentrations. In patients with acute and chronic liver diseases, protein C antigen concentration in plasma is positively correlated with serum albumin concentration and negatively correlated with the prothrombin time; the latter parameter has a sensitivity virtually identical to that of protein C as an indicator of liver synthetic function. Similar values of prothrombin time ratios and serum albumin concentration, chosen as a marker unrelated to blood coagulation, were found in survivors and nonsurvivors on Day 1.

Table 1. Plasma Protein C Antigen and Fibrinopeptide A Concentrations in Patients With Stroke by Lesion Pathology and Outcome

<table>
<thead>
<tr>
<th></th>
<th>Protein C antigen (%)</th>
<th>Fibrinopeptide A (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>n Mean CI</td>
<td>Mean CI</td>
</tr>
<tr>
<td>All stroke patients</td>
<td>37 104 92–118</td>
<td>7.1 5.1–9.7‡</td>
</tr>
<tr>
<td>Ischemic</td>
<td>29 103 92–115</td>
<td>7.2 5.0–10.4‡</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>8 107 70–164</td>
<td>6.5 3.5–11.9‡</td>
</tr>
<tr>
<td>Survivors</td>
<td>26 120 110–130*</td>
<td>4.8 2.9–7.9‡</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>11 75 55–102*</td>
<td>9.5 5.6–16.1‡</td>
</tr>
<tr>
<td>Controls</td>
<td>37 108 100–117</td>
<td>1.9 1.4–2.5</td>
</tr>
</tbody>
</table>

%: percent of pooled normal plasma; mean, geometric mean; CI, 95% confidence intervals; controls, patients with nonvascular neurologic disease.

*p < 0.05; †p < 0.01; ‡p < 0.001 different from controls.
A study was conducted to evaluate the protein C concentration in patients with acute stroke. The size of cerebral lesion on CT scan appeared negatively associated with survival. The observed mortality was 7% (1 of 14) in the class of stroke patients with small lesions (the one nonsurvivor died before Day 5 and had only one CT scan, that performed on admission), 30% (3 of 10) in the class of stroke patients with medium lesions, and 54% (7 of 13) in the class of stroke patients with large lesions. To evaluate whether the low plasma protein C concentration observed in nonsurvivors was related to the high percentage of stroke patients with large lesions in this subgroup (64%), protein C antigen concentrations in survivors and nonsurvivors were analyzed according to the size of the lesion (Figure 1). In patients with ischemic stroke, protein C concentration was significantly lower in the eight nonsurvivors than in the 21 survivors (73%, 58–91% vs. 115%, 105–126%, p < 0.01), with no apparent relation to the size of the lesion (Figure 1A). The three nonsurvivors in the subgroup of patients with hemorrhagic stroke all had large lesions and protein C antigen levels of 164%, 116%, and 27% (Figure 1B).

Survivors of stroke had plasma protein C antigen levels significantly higher than controls (p < 0.05, Table 1). During the early stages of disseminated intravascular coagulation, reduced protein C activity relative to antigen levels has been reported, with the suggestion that the discrepancy might be due to immunoreactive complexes of activated protein C with its specific inhibitor. To explore the possibility that the slightly increased protein C concentrations observed in survivors of stroke together with high FPA concentration might be due to the presence in plasma of inactive, complexed activated protein C, the anticoagulant and amidolytic activities of protein C were measured on admission and compared with protein C antigen concentrations. No discrepancy was observed in protein C concentrations measured with the three assays in 16 survivors (mean protein C anticoagulant activity 120%, 104–134%; mean protein C amidolytic activity 124%, 110–140%; mean protein C antigen concentration 117%, 103–136%).

In 17 survivors (two with hemorrhagic stroke) protein C antigen and FPA concentrations were also measured on Days 5, 15, and 60. No change in plasma protein C concentration was observed during follow-up (Figure 2). FPA concentrations were still high on Days 5 and 15 (p < 0.01), but they were similar to those found in the controls on Day 60 (2.66, 1.57–4.19 ng/ml; not significant) (Figure 2).

**Discussion**

Abnormalities of platelets, coagulation, and fibrinolysis have been described in patients with acute stroke. In our series of patients a state of activation of blood coagulation was present, as demonstrated by the high concentrations of FPA (a sensitive marker of in vivo thrombin activity) and was most probably secondary to the cerebrovascular event since FPA concentration was comparably elevated in patients with hemorrhagic and ischemic stroke. In a larger study that employed a battery of 13 tests to explore hemostasis, no parameter (isolated or in combination) discriminated hemorrhagic from ischemic stroke, indicating that activation of blood coagulation is a con-
sequence of the cerebral insult rather than a factor predisposing to the thrombotic event.\textsuperscript{15}

FPA concentrations were higher in nonsurvivors than in survivors (those who survived the 2 months' follow-up). Conversely, the mean plasma concentrations of protein C were slightly higher than normal in survivors but lower than normal in nonsurvivors. Although arterial thrombosis has been reported in patients congenitally deficient in protein C,\textsuperscript{8} little is known about the role of protein C in arterial occlusive disease and more specifically in cerebrovascular disease. High concentrations of protein C have been observed in patients with diabetes,\textsuperscript{28} ischemic cardiovascular disease,\textsuperscript{29,30} the nephrotic syndrome,\textsuperscript{31,32} and following the use of oral contraceptives,\textsuperscript{33} conditions with an increased risk of thrombosis and generally associated with elevated plasma concentrations of other vitamin K-dependent clotting factors.\textsuperscript{34,35} The survivors in our series of stroke patients had high antigenic and functional protein C concentrations, which were stable for 2 months after the event and appeared unrelated to the changes in FPA concentration in plasma. Since protein C is not an acute-phase reactant,\textsuperscript{36} it is likely that the high concentrations observed in survivors may have existed before the cerebrovascular event. The significance of increased protein C concentration in these patients remains to be established.

Nonsurvivors had significantly lower protein C concentrations than survivors, in some cases below the normal range, with no apparent relation to the size of the cerebral lesion. Although liver synthetic function was virtually identical and apparently normal in both subgroups of stroke patients, the possibility of low protein C concentrations existing before the acute event in nonsurvivors cannot be ruled out. However, it is more likely that a reduction in protein C concentration might have followed extensive activation of blood coagulation. Once activated by thrombin bound to the endothelial protein thrombomodulin, protein C is transformed into a serine protease with a relatively short half-life, estimated in animals to be 5–15 minutes.\textsuperscript{18} In disseminated intravascular coagulation the amount of activated protein C does not generally exceed 2–3% of the total circulating zymogen;\textsuperscript{35} however, serial time course studies conducted on patients with this condition have shown a decrease to 30–40% of the originally normal plasma concentrations of protein C antigen within 24–48 hours.\textsuperscript{18} Within 24–48 hours after the cerebrovascular event nonsurvivors had a mean protein C antigen concentration 63% of that in survivors. We speculate that in survivors of stroke, the relatively moderate formation of thrombin documented by lower FPA concentrations does not cause marked activation of protein C, thus resulting in no significant decrease of the originally elevated plasma concentrations. On the contrary, the higher concentration of thrombin attained in the circulation of nonsurvivors, as documented by their higher FPA concentrations, would lead to significant in vivo formation of thrombin-thrombomodulin complex, thus causing marked protein C activation and resulting in lower protein C concentrations. At the site of the lesion, however, the protective mechanism offered by the protein C system might be either ineffective or overcome by excessive formation of procoagulants, with fibrin deposition contributing to the extension of brain damage and eventually leading to death. The recent demonstration that thrombomodulin antigen is absent in the endothelium of human brain\textsuperscript{36} is not in contrast with this hypothesis. Assuming that thrombomodulin activity is also absent in the cerebral microcirculation, then thrombin generation at the site of the lesion would proceed uncontrolled by local activation of protein C. Under these conditions, the thrombin formed would complex with thrombomodulin in the pulmonary circulation, and virtually no activated protein C would be available to prevent the extension of cerebral damage.

Replacement of protein C has been suggested as a method to slow or stop the thrombotic process during disseminated intravascular coagulation.\textsuperscript{37} Experiments in which human activated protein C was infused into baboons have shown that activated protein C can function as an in vivo anticoagulant without suppressing factors V or VIII and that it can inhibit the factor V consumption that occurs following Escherichia coli infusion.\textsuperscript{37} Early supplementation with protein C might represent a safe therapeutic tool to stop the progression of cerebral damage in the acute phase of ischemic stroke.

In conclusion, our report strongly suggests the involvement of the protein C anticoagulant pathway in stroke and provides a rationale for clinical trials evaluating the therapeutic supplementation with protein C of patients with acute ischemic stroke. Despite the clearly different plasma protein C concentrations observed in survivors and nonsurvivors, individual
protein C antigen concentrations were within the normal range in the large majority of patients. High protein C concentrations may represent a favorable independent prognostic factor in acute stroke; however, the predictive value of this measurement appears limited.

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References


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