A cut stroke is an atherothrombotic or thromboembolic process in at least 80% of cases.\textsuperscript{1-3} Standard laboratory tests lack sensitivity for detecting or monitoring thrombosis and fibrinolysis in stroke. Recently developed assays specific for intermediate breakdown products (hemostatic markers) of fibrin formation and fibrinolysis allow us to assess better the hemostatic system in thrombotic diseases such as stroke. Recent work using these markers has suggested that peak endogenous fibrinolytic activity does not occur acutely following stroke.\textsuperscript{4,5} The aim of our study is to characterize the time course of thrombosis and fibrinolysis following acute ischemic stroke.

**Subjects and Methods**

Blood samples were obtained from 31 patients within 4 weeks of acute ischemic stroke (acute stroke patients, 14 men and 17 women, mean±SD age 69.8±10.6 [range 45–89] years). In 23, we obtained the initial sample during the first week following symptoms (Week 1); in 19, we obtained additional samples during the 4 weeks following stroke. Strokes were classified according to standard clinical and radiologic criteria as atherothrombotic, cardioembolic, or lacunar.\textsuperscript{3,6} Strokes in which no etiology was determined despite complete evaluation and those that fulfilled criteria for more than one category were classified as strokes of uncertain cause. At the time of admission, seven patients were receiving aspirin, three warfarin, and 21 patients no antiplatelet or anticoagulant therapy; one patient was placed on heparin shortly after admission. An index of stroke volume was obtained by multiplying the maximum width, maximum length, and maximum height of the hypodense area on a computed tomogram (CT scan) performed at least 3 days after stroke onset. Stroke severity in these 31 acute stroke patients was evaluated on admission using the Toronto Stroke Scale.\textsuperscript{7} An initial CT scan was used to rule out hemorrhagic strokes or other intracerebral processes. No acute stroke patient had a malignancy or was within 3 months of acute myocardial infarction or surgery.

Blood samples were obtained from 13 stable patients who were >3 months (range 3.1 months–6.4 years) after an acute ischemic stroke (chronic stroke patients, six men and seven women, mean±SD age 65±17 [range 24–89] years). The same exclusion criteria were used as for the acute stroke patients.

Blood samples were obtained from 13 healthy adults over age 50 who were without a history of cerebrovascular disease, coronary artery disease, peripheral vascular disease, valvular heart disease, or malignancy and who were not receiving antiplate-
TABLE 1. Hemostatic Markers of Fibrin Metabolism After Acute Stroke

<table>
<thead>
<tr>
<th>Marker</th>
<th>Acute stroke patients</th>
<th>Controls</th>
<th>Chronic stroke patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1 (n=23)</td>
<td>Week 2 (n=14)</td>
<td>Weeks 3–4 (n=13)</td>
</tr>
<tr>
<td>Fibrinopeptide A (ng/ml)</td>
<td>14.3±2.5*</td>
<td>11.6±3.0*</td>
<td>10.1±2.13*</td>
</tr>
<tr>
<td>B-β 1-42 peptide (pmol/ml)</td>
<td>5.7±0.40</td>
<td>5.1±0.30</td>
<td>5.5±0.50</td>
</tr>
<tr>
<td>B-β 15-42 peptide (pmol/ml)</td>
<td>22.0±1.80</td>
<td>24.2±5.80</td>
<td>35.7±10.6</td>
</tr>
<tr>
<td>Crosslinked D-dimer (ng/ml)</td>
<td>220±25.9*</td>
<td>363.9±94.3*</td>
<td>364±94*</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*p<0.05 different from control by Student's two-tailed unpaired t test.

let or anticoagulation therapy (controls, three men and 10 women, mean±SD age 62.4±8.7 years).

Informed consent was obtained from each patient or his or her legal representative. After discarding the first 4–5 ml, blood was collected by careful venipuncture and immediately placed into a pre-chilled tube containing an anticoagulant solution of 1,000 units/ml heparin, 900 kIU/ml aprotinin, and 40 mM ethylenediaminetetraacetic acid at a volume ratio of 9:1 blood:anticoagulant. The tubes were maintained in an ice bath for 15 minutes, then centrifuged at 1,500g for 20 minutes. Plasma was divided into aliquots and frozen at −40°C.

Aliquots of plasma for determination of fibrinopeptide A (FpA) concentration was treated with bentonite to remove fibrinogen and frozen at −40°C until assayed.* FpA was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (American Bioproducts, Parsippany, New Jersey). Absorbance at 492 nm was read with a Titertek plate reader (Flow Laboratories, Rockville, Maryland). Levels above the upper limit of the standard curve were assayed again using serial dilutions. Mean±SD concentration of FpA for normal subjects in our lab was 3.0±2.2 ng/ml. Concentrations of crosslinked D-dimer (XDP) in plasma were determined using a commercial ELISA kit (American Diagnostica, Inc., Greenwich, Connecticut). Mean±SD concentration of XDP for normal subjects was 69.7±30.6 ng/ml. Fibrinogen was removed from plasma aliquots for the determination of B-β 1-42 peptide (B-β 1-42) concentration by centrifuging in a Centricon-30 micro-concentrator (Amicon, Danvers, Massachusetts) at 1,500g for 60 minutes at 4°C. The fibrinogen-free ultrafiltrate was frozen at −40°C until assayed. Plasma aliquots for the determination of B-β 15-42 peptide (B-β 15-42) concentration were heated at 56°C for 30 minutes, centrifuged to remove the denatured fibrinogen, and frozen at −40°C until assayed. Both peptides were measured with commercial ELISA kits (New York Blood Center, New York, New York). Mean±SD concentrations of B-β 1-42 and B-β 15-42 for normal subjects were 6.7±3.2 nM and 21.8±5.3 nM, respectively.

The concentrations of FpA, B-β 1-42, B-β 15-42, and XDP for both patient groups and for each sampling time within the acute stroke patients group were compared with controls using Student’s two-tailed unpaired t test.

Results

Mean±SEM concentrations of FpA, B-β 1-42, B-β 15-42, and XDP for all three groups are listed in Table 1 and are depicted for the acute stroke patients and controls in Figures 1–4. Concentrations in blood samples collected within 48 hours after a stroke did not differ significantly from those collected during the remainder of the first week.

FpA levels in the acute stroke patients were elevated in 10 of 23 patients during Week 1 and in 10 of 19 patients with nonlacunar strokes; markedly elevated levels (>30 ng/ml) were seen in five of the 19 acute stroke patients with nonlacunar strokes. FpA levels declined from the initial level but remained significantly elevated throughout the 4 weeks after stroke (Figure 1). Six of the 13 acute stroke patients examined during Weeks 3–4 had elevated FpA levels.

In contrast, mean XDP levels in acute stroke patients were twice control during Week 1 and rose later (Figure 2). The difference in XDP concentration between Weeks 1 and 2 approached significance (p=0.08). Although 12 of 23 patients had elevated XDP concentrations during Week 1,
only one patient had a markedly elevated (>600 ng/ml) value.

Values of B-β 1-42 and B-β 15-42 in the acute stroke patients were not significantly different from control at any time, although there was a modest increase in the concentration of B-β 15-42 during Weeks 3 and 4 (Figures 3 and 4).

The ratio of the concentrations of FpA to B-β 1-42 is expressed as a multiple of the control ratio (1.0) (Figure 5). The ratios of the concentration of FpA to the other hemostatic markers, B-β 15-42 and XDP, are similarly shown in Figure 6.

There was no significant difference in concentrations of hemostatic markers among acute stroke patients with cardioembolic stroke (n=8), atherosclerotic stroke (n=8), or stroke of unknown cause (n=9). Values for all hemostatic markers were normal in acute stroke patients with lacunar infarcts (n=6) at all times following stroke.

Aspirin intake did not significantly affect the concentrations of these hemostatic markers. The three acute stroke patients receiving warfarin had elevated FpA concentrations that were similar to those of the other acute stroke patients, but the number of patients was too small for meaningful statistical analysis. The acute stroke patient who received heparin had an elevated FpA level (24 ng/ml) on admission before initiation of heparin therapy and normal FpA levels on Days 6 and 13.

Regression analysis showed no significant association between FpA levels and stroke volume (data not shown) with or without the inclusion of data from acute stroke patients with lacunar strokes. We also assessed initial stroke severity and found no relation to peak FpA levels or other markers.

Discussion

The acute manifestations of ischemic stroke are atherothrombotic or thromboembolic in at least 80% of cases.1-3 Angiography within 12 hours of acute strokes shows vascular stenosis or occlusion appropriate to the deficit in 80–90% of patients.9
Recognition of the thrombotic nature of most strokes has now led to therapeutic trials of fibrinolytic agents in acute stroke similar to their use in myocardial infarction. To better use these therapeutic agents, it is important to understand the natural thrombotic and fibrinolytic processes that occur in stroke.

The formation and breakdown of fibrin is shown schematically in Figure 7. The conversion of fibrinogen to fibrin by thrombin is a multistep process. Fibrin I is formed when FpA, a 16-amino-acid peptide, is cleaved from fibrinogen. Further thrombin action leads to the formation of fibrin II by the removal of another fragment, fibrinopeptide B. Finally, covalent bonds are formed between the chains producing fibrin polymer in a step requiring thrombin and Factor XIIIa.

Fibrinolysis depends on the conversion of plasminogen to the active enzyme plasmin, which is capable of degrading fibrinogen, fibrin I, and fibrin II. Plasmin is derived from the proenzyme plasminogen by a group of activators that includes tissue-type plasminogen activator and urokinase. Breakdown of each intermediate product yields specific peptide fragments; for instance, degradation of fibrin I and fibrinogen produces B-β 1-42, while degradation of fibrin II produces B-β 15-42. XDP is a specific fibrin polymer degradation product. The relative activities of thrombin and plasmin determine whether a specific intermediate product is degraded or proceeds to fibrin polymer formation.

Our data provide additional evidence that during the acute phase of ischemic stroke, thrombin activity (fibrin formation) greatly outweighs plasmin activity (fibrinolysis). This is clearly shown by the ratio of FpA to B-β 1-42 (Figure 5), which has been used as an index of thrombin activity relative to plasmin activity. The ratios of FpA to the other markers of fibrinolysis (Figure 6) were also elevated during Week 1.

During Week 2, fibrinolysis increased, as shown by rising XDP levels (Figure 2). However, even then no significant breakdown products of the intermediate species (B-β 1-42 or B-β 15-42) were measured (Figures 3 and 4). Since XDP is specific for plasmin degradation of fibrin polymer, this suggests that continued brisk thrombin activity drives the fibrin metabolism pathway to completion, and any plasmin present is able to act only on this end product.

Fisher and Francis also found a marked increase of FpA concentration compared with B-β 1-42 concentration and concluded that fibrinolytic mechanisms may be "impaired" in the cerebral circulation. In an earlier series by Lane et al., acute stroke patients had similar elevations of FpA and B-β 1-42 during Week 1. However, they did not measure B-β 1-42 concentration directly but extrapolated it from a measurement of thrombin-increase fibrinopeptide B immunoreactivity; we used an ELISA specific for B-β 1-42. Our simultaneous measurement of concentrations of other markers of fibrinolysis provides additional evidence of an increased relative activity of thrombin.

The lack of fibrinolytic response may not be specific for cerebrovascular thrombotic disease. Studies in patients with coronary artery disease have shown a similar divergence between thrombin activity and fibrinolytic activity. This has been ascribed to both deficient levels of plasminogen activators and to the presence of plasminogen activation inhibitors. Only a few studies of few patients have evaluated plasminogen activators or inhibitors in stroke, with conflicting results. Further studies that look specifically at tissue plasminogen activator activity and plasminogen activator inhibitors are necessary.
Concentrations of hemostatic markers were not elevated in acute stroke patients with lacunar stroke at any time following stroke. This is parallel to the finding of Shah et al, who found elevated levels of the platelet release proteins β-thromboglobulin and platelet factor 4 in atherothrombotic and cardioembolic stroke but not in lacunar stroke. It is not clear whether the inability to detect elevated fibrin marker concentration in these patients is related to the small size of the infarct or to a fundamental difference in pathophysiology. However, we found no relation between stroke volume and FpA levels. FpA concentration remained elevated for up to 4 weeks following acute ischemic stroke (Figure 1). Because the half-life of FpA is 3–5 minutes, this shows that thrombin activity persists for a prolonged period in many patients. Our data are in marked contrast to those from patients with acute myocardial infarction, in whom FpA levels decline to normal in approximately 24 hours. This suggests that this prolonged thrombin activity is a property of stroke and not thrombosis in general.

Elevated FpA levels were seen in recuperating outpatients returning for their clinic visits, suggesting that these levels were not related to complications of the stroke. Persistent elevation was independent of the type of stroke and occurred in patients in whom no etiology of stroke was found after complete investigation. Recurrent cerebral infarction, which might be expected if thrombus continued to form at the original site, was not seen. Thus, we do not believe that this explanation is likely.

Spontaneous lysis of clot has been shown to occur in stroke. It is possible that distal migration of clot could generate more thrombin activity. However, these angiographic changes occur early in the course of stroke and would not seem to explain the persistent elevation of FpA concentration.

Infarcted brain itself might activate thrombin activity. Brain thromboplastin is a powerful procoagulant. Blood–brain barrier breakdown as demonstrated by contrast enhancement of CT and radioisotope scanning often reaches a maximum 2–4 weeks after stroke, which approximates the time course we identified. Perhaps the leakage of brain tissue components into the vascular system (blood–brain barrier breakdown) may provide an ongoing stimulus for thrombin generation.

By assaying the concentrations of molecular markers of hemostasis, we have clarified the time course of thrombosis and fibrinolysis in patients with acute ischemic stroke. During the acute period, fibrin formation greatly exceeds fibrin degradation. Evidence of fibrinolysis develops slowly after stroke. The prolonged elevation of FpA concentration suggests that thrombin activity and fibrin formation persist for an extended period after stroke. Our findings have clinical and therapeutic implications.

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