Risk of Ischemic Stroke Associated With Functional Thrombin-Activatable Fibrinolysis Inhibitor Plasma Levels

A. Santamaría, MD; A. Oliver, MD; M. Borrell, PhD; J. Mateo, MD, PhD; R. Belvis, MD; J. Martí-Fàbregas, MD, PhD; R. Ortín; I. Tirado, PharmD; J.C. Souto, MD, PhD; J. Fontcuberta, MD, PhD

Background and Purpose—Recently, a novel procarboxypeptidase B–like proenzyme, called thrombin-activatable fibrinolysis inhibitor (TAFI), has been described. It plays an important role in the delicate balance between coagulation and fibrinolysis. TAFI leads to potent inhibition of tissue plasminogen activator–induced fibrinolysis. The relevance of TAFI in thromboembolic disease is unclear. We have investigated the risk of ischemic stroke (IS) in relation to plasma levels of functional TAFI.

Methods—In a case-control study, we enrolled 264 individuals; 114 had IS, and 150 were recruited as controls who were age and sex matched and had no history of arterial disease. The individuals supplied information on their personal and family histories of cardiovascular diseases and conventional cardiovascular risk factors. Functional TAFI assays were performed by use of a method based on the activation of TAFI with thrombin-thrombomodulin and the measure of the TAFI activity generated. Other hemostatic parameters assayed were factor VIIIc, anti-phospholipid antibodies, fibrinogen, factor V Leiden, and the prothrombin gene G20210A mutations (PT20210A).

Results—Functional TAFI levels were significantly higher in patients with IS (113.7 ± 25%; range, 57% to 209%) than in controls (102.6 ± 19%). The odds ratio for IS in patients with functional TAFI levels >120% was 5.7 (95% confidence interval, 2.3 to 14.1).

Conclusions—We found that functional TAFI levels in plasma (>120%) increased the risk of IS ≈6-fold. Further studies should elucidate the physiological role of TAFI in arterial disease and possibly provide clues to therapeutic approaches.

Key Words: fibrinolysis stroke ischemic thrombosis

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teroidal thrombotic disease is the most frequent cause of morbidity and mortality in developed countries.1 There is good evidence that an imbalance in the hemostatic system can lead to arterial thrombosis.1,2 The arterial thrombotic diseases such as ischemic stroke (IS) arise from 2 processes: atherosclerosis and thrombosis. Research on the nature of the interaction between hemostatic factors and the endothelial cell surface of blood vessels should help to identify risk factors for arterial disease and individuals at increased risk.

Deficiencies in or increased levels of some hemostatic factors such as fibrinogen and factor VIIIc have been associated with increased risk of arterial thrombotic disease. However, the results of some studies are controversial.1–5 It is known that impaired fibrinolytic system factors are involved in the pathogenesis of atherosclerotic disease.2

A novel procarboxypeptidase B–like proenzyme, called thrombin-activatable fibrinolysis inhibitor (TAFI), has been described recently.6–7 It plays an important role in the delicate hemostasis balance between coagulation and fibrinolysis. TAFI is converted to an active carboxypeptidase (TAFIa) by thrombin, plasmin, trypsin, and more efficiently the thrombin-thrombomodulin complex.8–10 Activated TAFI inhibits fibrinolysis by removing carboxy-terminal lysine residues that appear during proteolysis of the fibrin polymers during the process of clot lysis. These residues are important for binding and activating plasminogen. Thus, TAFI leads to a potent inhibition of tissue plasminogen activator–induced fibrinolysis.8–11 It is clear that plasma TAFI levels are major determinants of clot lysis time because of the downregulation of plasminogen activation and fibrinolysis.12 Functional TAFI, described by Mosnier et al.,11 is based on the activation of TAFI with thrombin-thrombomodulin and the measure of the generated TAFI activity.

Although the precise relevance of TAFI in thromboembolic diseases is unclear, high plasma levels of TAFI may be related to thrombotic disorders. For example, reports have indicated that elevated antigenic TAFI levels constitute a mild risk factor for venous thrombosis.12–14 Only a few studies have investigated the role of TAFI levels in coronary arterial disease, suggesting that an increase in antigenic TAFI

Received January 30, 2003; final revision received May 13, 2003; accepted June 3, 2003.

From the Hemostasis and Thrombosis Unit, Hematology Department (A.S., M.B., J.M., R.O., I.T., J.C.S., J.F.), Neurology Department, (R.B., J.M.-F.), and Fundación Puigvert (A.O.), Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Correspondence to Dr A. Santamaría, Department of Hematology, Hospital de la Santa Creu i Sant Pau, C/ Sant Antoni Ma Claret, 167, 08025-Barcelona, Spain. E-mail msantamaria@hsp.santpau.es

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*Stroke* is available at [http://www.strokeaha.org](http://www.strokeaha.org) DOI: 10.1161/01.STR.0000088642.07691.15

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Atrial fibrillation was recorded on the basis of ECG findings by a cardiologist. If they were previous or current smokers of 5 cigarettes per day, morbid obesity was defined as a body mass index (BMI) of at least 30 kg/m². Individuals were considered smokers for fibrinogen. Anti-phospholipid antibodies were considered positive if the fibrinogen levels. Cutoffs were 170% for factor VIII and 3.1 g/L for fibrinogen. We converted into binary variables the factor VIII plasma levels and calculated ORs. To determine the adjusted OR for different variables, contingency tables. Receiver-operating characteristic curves were used to determine the cutoff value of functional TAFI. We used the cutoff of 120% of functional TAFI levels as a reference group to calculate ORs. To determine the adjusted OR for different variables, we converted into binary variables the factor VIII plasma levels and the fibrinogen levels. Cutoffs were 170% for factor VIII and 3.1 g/L for fibrinogen. Anti-phospholipid antibodies were considered positive when one was higher than the normal range or when the lupus anticoagulant test was positive.

Levels may be a risk factor, although this association has not been confirmed in other studies. To the best of our knowledge, no studies have investigated the relation of functional levels of TAFI with IS. Here, we report the results of our investigation into the association of plasma levels of functional TAFI with IS. It is based on the activation of TAFI in plasma with the thrombin-thrombomodulin complex and the measure of activated TAFI with hippuril-arginine substrate. Anti-phospholipid antibodies, including anti-phosphatidylserine and anti-cardiolipin IgG and IgM, were measured by in-house enzyme-linked immunosorbent assay anti-cardiolipin and anti-phosphatidylserine method as described by Gharavi et al. Anti-cardiolipin IgG <13.97 GPL, anticardiolipin IgM <25.32 MPL, anti-phosphatidylserine IgG index <2.8, and anti-phosphatidylserine IgM index <3.7 were considered normal values. Lupus anticoagulant was performed by the Exner method. Fibrinogen was measured by the von Clauss method. Factor V Leiden (FVL) and prothrombin gene G20210A mutations (PT20210A) were analyzed as described previously.

**Methods**

**Patients and Control Subjects** A total of 264 consecutive individuals <80 years of age were included in our case-control study; 114 were admitted to the Neurology Unit in the Hospital de la Santa Creu i Sant Pau with the diagnosis of IS, and 150 individuals (spouses and friends of patients) served as controls.

Patients assigned to the IS group had at least 1 episode of IS, including established or transient ischemic cerebrovascular attack, excluding patients with unusual causes of IS. The diagnosis of IS was based on findings of general clinical and neurological examination and at least 1 objective diagnostic method such as CT or MRI of the brain in all patients.

Control subjects had no previous history of thromboembolic disease (including venous and arterial thrombosis) and were matched to the patients in age and sex. Informed consent was obtained from all participants. The interview included questions on personal and family histories of cardiovascular diseases and conventional cardiovascular risk factors. We considered conditions like hypertension, diabetes, and dyslipidemia only if the individual had been diagnosed by a physician or was currently taking prescription drugs for any 1 or more of these conditions. Alcohol intake of >300 g/wk was considered significant intake. Morbid obesity was defined as a body mass index (weight in kilograms divided by height squared in meters) of at least >30 kg/m². Individuals were considered smokers if they were previous or current smokers of >5 cigarettes per day. Atrial fibrillation was recorded on the basis of ECG findings by a cardiologist.

**Laboratory Methods** Blood samples were obtained from the antecubital vein at least 1 month after the acute thrombotic episode. Whole-blood samples for hemostatic tests were collected in 1/10 volume of 0.129 mol/L sodium citrate as the anticoagulant. Assays for factor VIII were performed immediately on fresh plasma samples. The remaining plasma samples were stored at −80 °C until used. Factor VIII was assayed with deficient plasma from Diagnostica Stago (Asnières). Plasma functional TAFI was measured with the method described by Mosnier et al. It is based on the activation of TAFI in plasma with the thrombin-thrombomodulin complex and the measure of activated TAFI with hippuril-arginine substrate. Anti-phospholipid antibodies, including anti-phosphatidylserine and anti-cardiolipin IgG and IgM, were measured by in-house enzyme-linked immunosorbent assay anti-cardiolipin and anti-phosphatidylserine method as described by Gharavi et al.

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**Statistical Analysis** Statistical analyses were performed by conventional software. Values are expressed as mean±SD. Student’s t test was used to calculate the mean differences between groups. Odds ratios (ORs) were calculated as relative risk for thrombosis in the unmatched fashion adjusted for age and sex, and other covariables were calculated by conditional logistic regression analysis. Confidence intervals (CIs) were derived from the model. Unadjusted ORs were obtained from contingency tables. Receiver-operating characteristic curves were used to determine the cutoff value of functional TAFI. We used the cutoff of 120% of functional TAFI levels as a reference group to calculate ORs. To determine the adjusted OR for different variables, we converted into binary variables the factor VIII plasma levels and the fibrinogen levels. Cutoffs were 170% for factor VIII and 3.1 g/L for fibrinogen. Anti-phospholipid antibodies were considered positive when one was higher than the normal range or when the lupus anticoagulant test was positive.

**Results**

The basic characteristics of the sample population are given in Table 1. Mean age at the time of the first episode of IS was 56 years (range, 23 to 80 years); mean age of the controls was higher than the normal range. Results of laboratory tests are given in Table 2. Table 3 shows the results of logistic regression analysis, and the statistical significance of the associations is indicated. The adjusted ORs were calculated by considering the influence of other variables. The adjusted ORs were calculated by considering the influence of other variables.
TABLE 2. Distribution of Functional TAFI Plasma Levels (%) Related to Age and Sex in Cases and Controls

<table>
<thead>
<tr>
<th>Age Range, y</th>
<th>Cases</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>20–55</td>
<td>n 28</td>
<td>27</td>
</tr>
<tr>
<td>TAFI, %</td>
<td>114.6</td>
<td>110.8</td>
</tr>
<tr>
<td>56–80</td>
<td>n 34</td>
<td>24</td>
</tr>
<tr>
<td>TAFI, %</td>
<td>115.8</td>
<td>112.5</td>
</tr>
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48 years (range, 21 to 75 years). Fifty-two women and 62 men were in the IS group; 83 women and 67 men were in the control group. All of the major cardiovascular conventional risk factors such as smoking, dyslipidemia, hypertension, morbid obesity, and diabetes mellitus were associated with a significant increased risk of IS. Atrial fibrillation was significantly associated with the risk of IS (OR, 6.9; 95% CI, 1.9 to 24.5). In addition, as might be expected, family history of arterial disease and diabetes were the most relevant risk factors in patients with IS. Positive anti-phospholipids antibodies were found in 11.4% of cases compared with 6.3% of controls, but the difference was not statistically significant (OR, 1.9; 95% CI, 0.8 to 4.6). PT20210A and FVL mutations were present in 5.3% versus 3.3% and 0.9% versus 4% of the patients compared with controls, but the differences were not statistically different. Fibrinogen (mean, 3.8 g/L; range, 0.8 to 8.4 g/L) and factor VIIc (mean, 216%; range, 76% to 516%) were significantly increased in patients compared with controls (3.3 g/L; range, 2.1 to 7.7 g/L) and 154.4% (range, 42% to 328%), but the adjusted OR for fibrinogen was not increased significantly.

Functional TAFI levels were significantly higher in patients with IS (113.7 ± 25%; range, 57% to 209%) than in controls (102.6 ± 19%) (P < 0.05) (not shown in tables). Variations of mean values of functional TAFI levels with age and sex in both groups are shown in Table 2. Patients and controls were divided into 2 subgroups of age: a 20- to 55-year-old group and a 56- to 80-year-old group. In the control group, there were no differences between younger and older groups in either women or men. In the patient group, there were also no statistically significant differences between groups by age in either women or men. Younger women and men with IS showed higher levels of functional TAFI levels compared with women and men in the control group. Additionally, older men and women with IS showed higher functional TAFI levels compared with men and women of the control group. The correlation of functional TAFI levels and conventional cardiovascular risk factors is summarized in Table 3. We did not find any statistical differences in mean values of functional TAFI levels in either patients or controls related to different risk factors such as hypertension, diabetes, obesity, alcohol intake, dyslipidemia, or smoking. Only patients with a family history of arterial disease showed higher levels of functional TAFI levels than patients without a family history (118.1% and 108.1%, respectively; P < 0.05).

To determine the risk of IS, we used the cutoff level of 120% for functional TAFI (Table 4). Unadjusted and adjusted ORs were calculated using as the reference group patients with TAFI levels <120%. However, the unadjusted OR for functional TAFI levels was 5.8 (95% CI, 3.1 to 11.5) in IS patients compared with controls. Furthermore, when we analyzed risk adjusted by sex, age, and other putative confounding variables like factor VIIIc, smoking, dyslipidemia, hypertension, alcohol intake, diabetes, fibrinogen, anti-phospholipids antibodies, FVL, and prothrombin G20210A mutation, the adjusted OR remained as high as 5.7 (95% CI, 2.3 to 14.1) for IS patients with plasma functional TAFI levels >120%.

**Discussion**

In our case-control study, we found that a high level of functional TAFI in plasma engenders a significant risk for IS. Thus, functional TAFI levels in plasma >120% increased the risk of IS ~6-fold. It is remarkable that the risk did not change even when adjusted for confounding variables, including major conventional cardiovascular risk factors and other factors such as high levels of factor VIII and fibrinogen. We found higher mean levels of functional TAFI levels in cases compared with controls. The variation of functional TAFI levels in controls and cases was not correlated with either sex or age. Mean functional TAFI levels were higher in different age groups in patients, both women or men, compared with different groups of controls. We did not find a significant correlation of functional TAFI levels with conventional cardiovascular risk factors. This lack of correlation may be related to a weak effect of these risk factors with

<table>
<thead>
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<th>Table 3. Correlation of Functional TAFI Levels With Conventional Cardiovascular Risk Factors</th>
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<tbody>
<tr>
<td>Functional TAFI (%), N/Y</td>
</tr>
<tr>
<td>Hypertension</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Alcohol intake</td>
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<td>Diabetes mellitus</td>
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<td>Family history of arterial disease</td>
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<td>Obesity</td>
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<td>Dyslipidemia</td>
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*Adjusted by age, sex, smoking, dyslipidemia, diabetes, family history of arterial disease, anti-phospholipids, PT20210A, and FVL mutations, fibrinogen, and factor VIII levels.
†Reference group.
modifications of functional TAFI levels. Only a family history of arterial disease was correlated with higher functional TAFI levels in patients than in the controls. Conventional cardiovascular risk factors were more prevalent in patients than in controls and were associated with a higher risk of IS. As in other studies, we did not find a higher risk of arterial thrombotic disease in patients with FVL or in those who were PT20210A carriers. Other established hemostatic risk factors such as high levels of factor VIII and fibrinogen also showed an increase risk of IS. Family history of IS was the most important risk factor associated specifically with the development of IS. To the best of our knowledge, this is the first case-control study that presents evidence that a high risk of IS is associated with high functional TAFI levels.

Although different studies confirm that TAFI is an important link between coagulation and fibrinolysis, the precise role of TAFI in arterial and venous diseases is unclear. Its mechanism of action and its relation with endothelium and its modulators may explain why high levels of TAFI are associated with a higher risk of IS. Our results clearly demonstrate that an increased risk of IS is related to functional plasma TAFI levels. One possible explanation is the influence of platelets, which contribute differently to the pathogenesis of arterial and venous thrombosis, as does the susceptibility of veins and arteries to atherosclerosis. Platelets may adhere to specific regions of the endothelium in response to injury and may be involved in the pathogenesis of inflammatory and thrombotic disorders. Platelets contain TAFI in α-granules, which is secreted during platelet activation. So, TAFI activation and TAFI secretion would depend on the generation of thrombin in a dose-dependent manner. High levels of TAFI may play an important role in protecting the plug against the fibrinolytic pathway, and the aggregated platelets would increase TAFI levels and the strength of the clots.

Because TAFI is activated with high efficiency by the thrombomodulin-thrombin complex, the thrombin generated and the thrombomodulin concentration on the endothelium are important regulators of TAFI activation. The thrombomodulin-thrombin complex can activate both protein C and TAFI, depending on its concentration. Thus, low concentrations of thrombomodulin enhance TAFI activation, whereas high concentrations of thrombomodulin increase activation of protein C, which decreases the thrombin generation and consequently decreases TAFI activation.

Small arterial vessels such as those in the brain or coronary arteries have lower concentrations of thrombomodulin compared with veins. Therefore, high levels of TAFI in some specific types of vessels with low concentrations of thrombomodulin (like arteries) may increase the downregulation of fibrinolysis and reduce the capacity to remove fibrin clots from the circulation. This would lead to an increased risk of IS.

Understanding the role of TAFI in IS may have clinical implications. Some studies using in vivo experiments in animals found that treatment with TAFI inhibitors (carboxypeptidase B inhibitor derived from potato, also known as PCI) added to recombinant tissue plasminogen activator increased the rate of clot lysis in physiological conditions. As a consequence, TAFI may be an important target for enhancing thrombolysis.

In conclusion, we have found an ≈6-fold (5.8) increased risk of IS associated with increased functional TAFI levels in plasma. Therefore, high TAFI levels may represent a significant risk factor for IS. Because it is a complex disease, knowledge of the pathophysiological role of different biological and genetic markers will lead to a better understanding of the mechanism of the thrombotic disease. Further studies should elucidate the physiological role of TAFI in arterial diseases and lead to possible therapeutic approaches.

Acknowledgment

We are indebted to Professor W.H. Stone for his help in the revision and discussion of the manuscript.

References


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Stroke. 2003;34:2387-2391; originally published online August 28, 2003;
doi: 10.1161/01.STR.0000088642.07691.15

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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