Thrombin-Activable Fibrinolysis Inhibitor Levels in the Acute Phase of Ischemic Stroke

Joan Montaner, MD, PhD; Marc Ribó, MD; Jasone Monasterio, MD, PhD; Carlos A. Molina, MD, PhD; José Alvarez-Sabín, MD, PhD

Background and Purpose—Thrombin-activable fibrinolysis inhibitor (TAFI) is a recently identified fibrinolysis inhibitor in plasma. The purpose of this work was to study TAFI levels in the acute phase of ischemic stroke and their relationship with stroke evolution.

Methods—In 30 consecutive ischemic stroke patients, TAFI plasma levels were measured by means of enzyme-linked immunosorbent assay (percentage of the pooled reference kit expressed as mean±SD) and compared with the values obtained in 30 healthy control subjects. All samples were drawn within the first 24 hours after symptom onset (mean, 4.6 hours) and before any treatment was started.

Results—TAFI plasma concentration was significantly higher \((P<0.001)\) in stroke patients \((158.4±53.2\%)\) than in healthy control subjects \((105.6±30.2\%).\) The highest TAFI levels were found in cases of neurological deterioration \((worsening, 198.1±63.0\%; stability, 130.5±39.3\%; improvement, 173.9±52.0\%; P=0.057).\)

Conclusions—High levels of TAFI are found in the acute phase of ischemic stroke. (Stroke. 2003;34:1038-1040.)

Key Words: carboxypeptidase U ▪ cerebral ischemia ▪ stroke ▪ TAFI ▪ thrombolysis

Thrombin-activable fibrinolysis inhibitor (TAFI), also known as procarboxypeptidase B, is a plasma zymogen that potently inhibits fibrinolysis\(^1\) when converted to an enzyme. The direct action of TAFI as an inhibitor of clot lysis involves removal of carboxy-terminal lysyl and arginyl residues from partially degraded fibrin.\(^2\) Consequently, plasminogen binding sites are eliminated, and plasminogen activation and fibrinolysis are inhibited.\(^3\) Accordingly, it has been hypothesized that increased TAFI activity is associated with a proneness to thrombosis.

In fact, elevated TAFI plasma levels have been found in men with symptomatic coronary artery disease\(^4\) and are a mild risk factor for deep vein thrombosis.\(^5\) Actually, no data are available on TAFI in stroke patients or experimental brain ischemia, a field in which TAFI overexpression might block thrombus resolution.

In this preliminary report, our aim was to determine total antigen TAFI levels in acute stroke patients and to study its relationship with stroke evolution.

Subjects and Methods

Study Population

We prospectively studied consecutive patients entering the emergency department with confirmed ischemic stroke. A total of 30 patients evaluated within 24 hours of symptom onset by the on-call neurologist were included in the study during September 2001. A detailed history of vascular risk factors was obtained from each patient. Etiological subgroups were defined according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.\(^6\)

Clinical Assessment

Patients were also classified according to the Oxfordshire Community Stroke Project (OCSP) clinical classification into 1 of 4 clinical syndromes: total anterior circulation infarcts, partial anterior circulation infarcts, lacunar infaracts, and posterior circulation infarcts.\(^7\) This information was used as an indirect measure of stroke extension.\(^8\) We assessed clinical status on patient arrival and discharge from hospital by means of the National Institutes of Health Stroke Scale (NIHSS). Neurological deterioration or improvement was defined as an increase or a decrease of >4 points in NIHSS score. Patients with a known inflammatory or malignant disease were excluded.

Control Group

Thirty healthy subjects with no history of thromboembolic events or bleeding tendency were studied. Mean age of the control group was 66.8 years (range, 58 to 83 years). Control subjects <65 years of age were recruited from the blood bank donors, and control subjects >65 years of age were relatives of the studied patients.

TAFI Immunoassay Methods

Blood samples were drawn on patient arrival at the hospital and before any treatment was started. EDTA tubes were used to collect blood. Plasma was immediately separated by centrifugation at 3000 rpm for 15 minutes and stored at \(-80°C\). Enzyme-linked immunosorbent assays were performed according to manufacturer’s instruction (Zymutest TAFI Ag, Hyphen BioMed) and validated in our laboratory. Results are expressed as a percentage of a control pooled plasma of healthy subjects provided with the kit. The mean intra-assay variation was <10% for both patients and control subjects.

Received May 20, 2002; final revision received October 16, 2002; accepted October 28, 2002.

From the Cerebrovascular Unit (J. Montaner, M.R, C.A.M, J.A.S) and the Vascular Biology and Hemostasia Unit (J. Montaner, J. Monasterio), Vall d’Hebron Hospital, Barcelona, Spain.

Correspondence to Dr Joan Montaner, Unidad Cerebrovascular, Servicio de Neurología, 6º Planta, Hospital General, Hospital Vall d’Hebron, Pg Vall d’Hebron 119-129, 08035 Barcelona, Spain. E-mail 31862jmv@comb.es

© 2003 American Heart Association, Inc.

Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000063139.06585.45

1038
Statistical Analysis

Descriptive and frequency statistical analyses were obtained, and comparisons were performed by use of the SPSS statistical package, version 10.0. Statistical significance for intergroup differences was assessed by Student’s t test and analysis of variance. Pearson’s and Spearman’s correlation coefficients were used to determine correlation between continuous and scale variables, respectively, and the χ² test was used to compare categorical variables. A difference of P<0.05 was considered statistically significant.

Results

The stroke patient group included 14 men and 16 women (46.6% and 53.4%, respectively). Mean patient age was 77.5 years (range, 45 to 95 years). According to TOAST criteria, 50% of patients had a cardioembolic stroke, 13.3% had an atherothrombotic stroke, and 13.3% experienced a lacunar stroke, whereas 23.3% were of undetermined origin. Attending to the OCSP classification, we found total anterior circulation infarcts in 30% of patients, partial anterior circulation infarcts in 56.6%, and lacunar infarcts in 13.3%.

Blood samples were obtained shortly after stroke symptom onset (mean, 4.6±4.3 hours), and no association was found between time elapsed from symptom onset to sample drawing and the TAFI plasma level (r=0.25, P=0.24).

Mean TAFI level in the stroke patient group (158.4±53.2%) was significantly (P<0.001) higher than in the control group (105.6±30.2%). This difference was found for almost every subtype of ischemic stroke, regardless of origin or extension (Figure 1).

TAFI level according to the presence of classic risk factors is shown in the Table. Dyslipidemia tended to be related to higher

| TAFI Levels According to the Presence of Classic Cardiovascular Risk Factors and for the Prior Use of Antiplatelet or Anticoagulant Drugs Among Stroke Patients |
|---------------------------------|-----------|-----------|--|
|                                | Yes       | No        | P  |
| Males                          | 164.2±59.9| 155.6±50.0| 0.67|
| (n=14)                         | (n=16)    |           |    |
| Prior ischemic stroke          | 138.2±57.5| 159.7±47.1| 0.32|
| (n=7)                          | (n=23)    |           |    |
| Peripheral arteriopathy        | 287.4     | 151.7±44.3| ...|
| (n=1)                          | (n=29)    |           |    |
| Ischemic cardiopathy           | 127.5±35.4| 161.8±51.8| 0.21|
| (n=4)                          | (n=26)    |           |    |
| Atrial fibrillation            | 170.4±46.5| 145.8±52.8| 0.21|
| (n=12)                         | (n=18)    |           |    |
| Hypertension                   | 153.3±58.2| 162.6±36.6| 0.65|
| (n=17)                         | (n=13)    |           |    |
| Diabetes mellitus              | 180.6±100.3| 151.3±33.2| 0.55|
| (n=5)                          | (n=25)    |           |    |
| Dyslipidemia                   | 197.2±63.0| 149.7±46.4| 0.08|
| (n=4)                          | (n=26)    |           |    |
| Heavy smokers                  | 140.6±40.6| 160.4±52.9| 0.44|
| (n=5)                          | (n=25)    |           |    |
| Alcohol abuse                  | 163.9±107.0| 155.9±43.6| 0.90|
| (n=3)                          | (n=27)    |           |    |
| Prior antiplatelet drugs       | 161.1±77.8| 162.0±51.8| 0.97|
| (n=6)                          | (n=24)    |           |    |
| Prior anticoagulant drugs      | 170.4     | 160.5±55.3| ...|
| (n=1)                          | (n=29)    |           |    |

TAFI antigen levels are given as mean±SD (%).
TAFI levels (197.2±63.0% versus 149.7±46.4%, P=0.08). No association was found between TAFI levels and age in either the patient group (r=0.18, P=0.33) or the control group (r=−2.94, P=0.11) (Figure 2). An association was found between TAFI level and leukocytes count at arrival (r=0.48, P=0.019).

The highest level of TAFI was found among patients who worsened (n=4; 198.1±63.0%; range, 141.7% to 287.4%) during the acute phase, although the difference with patients who remained stable (n=9; 130.5±39.3%; range, 52.2% to 193.2%) or improved (n=14; 173.9±52.0%; range, 105.6% to 277.6%) was not statistically significant (P=0.057). No association was found between baseline NIHSS and TAFI level (r=−0.12, P=0.52).

Discussion
TAFI is a recently identified fibrinolysis inhibitor in plasma and is considered to be an important link between coagulation and fibrinolysis. In this study, we found for the first time an increased level of TAFI in patients who suffered ischemic stroke.

Cardiovascular risk factors did not influence TAFI level among our stroke population, and only dyslipidemic patients tended to have higher concentrations of TAFI. Other authors also failed to demonstrate important relations of TAFI with cardiovascular risk factors. Because of the large interindividual variability of TAFI antigen level and the weak relationship with lifestyle characteristics, it has been suggested that TAFI antigen is mainly under genetic control.

TAFI is a proenzyme that, after activation by thrombin, thrombin/thrombomodulin, or plasmin, downregulates fibrinolysis. Thus, the coagulation and fibrinolytic processes are linked by virtue of thrombin-catalyzed activation of TAFI, and factors or conditions (ie, stroke) that influence thrombin generation also influence fibrinolysis via a TAFI-dependent mechanism. Moreover, inhibition of TAFI accelerates tissue plasminogen activator–induced clot lysis in vitro and enhances thrombolysis in animal models. Therefore, modulation of TAFI activity can have an important implication for the stability of fibrin clots.

Although very high levels of this carboxypeptidase were particularly evident in patients with neurological worsening during the first days, patients who improved also had high levels of TAFI. Thus, the attractive hypothesis in which the endogenous fibrinolytic activity directed to disrupt the clot may be blocked by a high TAFI concentration remains to be demonstrated.

Study Limitations
Our study design does not allow high TAFI level to be defined as a risk factor for stroke because TAFI production could also be a consequence of stroke. Although TAFI correlation with leukocytes suggests an acute-phase response, its lack of association with stroke extension does not support this point.

Other molecules involved in the mechanisms of excessive fibrin formation (eg, plasminogen activator inhibitor–1) have not been studied here. Moreover, we have determined TAFI antigen, but future studies aimed at testing TAFI activity might offer additional information.

Conclusions
High levels of TAFI are found in the acute phase of ischemic stroke. Whether this fact creates a hypofibrinolytic state that influences stroke outcome requires further investigation.

Acknowledgments
We are grateful to Manolo Quintana for statistical advice and to Dorita Quiroga and Pilar Bermudez for technical assistance.

References
Thrombin-Activable Fibrinolysis Inhibitor Levels in the Acute Phase of Ischemic Stroke
Joan Montaner, Marc Ribó, Jasone Monasterio, Carlos A. Molina and José Alvarez-Sabín

Stroke. 2003;34:1038-1040; originally published online March 20, 2003;
doi: 10.1161/01.STR.0000063139.06585.45
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/34/4/1038

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/