The Release of Tumor Necrosis Factor–α is Associated with Ischemic Tolerance in Human Stroke

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Tumor necrosis factor (TNF)–α overexpression has been related to experimental ischemic tolerance when transient ischemia precedes cerebral infarction. We investigated TNF-α and interleukin (IL)–6 plasma concentrations in 283 patients with an acute stroke within 24 hours after symptom onset. An ipsilateral transient ischemic attack (TIA) within 72 hours before stroke was recorded in 38 patients. The infarct volume measured on computed tomography on days 4 to 7 and the frequency of poor outcome (Barthel Index score < 85) at 3 months were significantly lower in patients with prior TIA. Plasma concentrations of TNF-α were higher (42.5 ± 9.9 vs 13.1 ± 6.4pg/ml, $p < 0.0001$) and IL-6 levels were lower (10.1 ± 6.2 vs 28.3 ± 17.3pg/ml, $p < 0.0001$) in patients with prior TIA. A new variable termed TNF-α/IL-6 index was considered positive when TNF-α was greater than 30pg/ml and IL-6 was less than 30pg/ml. Positive TNF-α/IL-6 index was found in 92% of patients with prior TIA and in 1% of those without. TNF-α/IL-6 index ($p = 0.0003$) and TIA ($p = 0.0001$) were associated with good outcome in logistic regression analysis after adjusting for potential confounding factors. Ischemic tolerance in acute stroke is associated with increased plasma levels of TNF-α in the presence of reduced concentrations of IL-6.

Ann Neurol 2003;54:811–819

In the last decade, a broad research has demonstrated that short periods of ischemia, whether local or global, but nonlethal (ischemic preconditioning), have a protective effect against later episodes of permanent cerebral ischemia (ischemic tolerance).[1,2] This phenomenon of ischemic tolerance previously was confirmed in the heart, both in experimental models[3] and in human clinical practice.[4] More recently, this fact has been postulated in patients with ischemic stroke preceded by ipsilateral transient ischemic attacks (TIAs) within a short time interval.[5–7] This time interval is crucial, because it has been demonstrated that when ipsilateral TIAs precede the current infarction by more than 72 hours, the outcome is similar to that of the patients with contralateral TIA or without previous TIA.[6,8]

The mechanisms induced by ischemic preconditioning and their relationships with the phenomenon of ischemic tolerance are not well understood.[9] Some studies have suggested the induction of protein synthesis with increased expression of immediate-response genes[10] or suppression of apoptotic genes,[11] activation of *N*-methyl-D-aspartate receptors[12] or of the A1 adenosine receptors.[13] More recently, the activation of nuclear factor–κB (NF-κB) has been implicated as responsible for the development of cerebral ischemic tolerance in animal models.[14–16] This factor has a crucial role in neuronal survival and is activated, among other mechanisms, by proinflammatory cytokines. Among these cytokines, tumor necrosis factor (TNF)–α has been implicated in brain damage but also in neuroprotective effects in stroke[16–19] (reviewed in Hallenbeck and colleagues 19), as we have demonstrated in rat cortical cultures exposed to oxygen-glucose deprivation or excitotoxic stimuli as well as in in vivo models of ischemic tolerance.[20,21]

Moreover, it has been shown in various experimental models that this ischemic preconditioning induces a reduction of the inflammatory response, and specifically, of the liberation of interleukin (IL)–6.[22,23] Our work attempts to study the role of TNF-α and IL-6 in a clinical model of patients with ischemic stroke with and without prior ipsilateral TIA, and their relationship with the phenomenon of ischemic tolerance.

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Received Mar 28, 2003, and in revised form Aug 11. Accepted for publication Aug 16, 2003.

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Patients and Methods

We studied 283 patients (142 women and 141 men; age [mean ± SD], 66.6 ± 10.9 years) with ischemic stroke selected from a total of 521 consecutively admitted in one hospital between September 1999 and October 2001. The patients were included in a prospective stroke data bank of patients with diagnoses of hemispheric cerebral infarcts within 24 hours from the onset of symptoms. The aim of the survey was to confirm our preliminary data concerning the more satisfactory clinical and radiological outcome in patients who present with ipsilateral TIA within the previous 72 hours,[6,8] and to study the biochemical markers implicated in this phenomenon. Exclusion criteria, for the purpose of this research, were established after the completion of the data bank. Reasons for exclusion were the diagnosis of lacunar infarction in 56 patients, lack of stored blood samples in 29 patients, thrombolytic treatment with intravenous t-PA in 11 patients, inclusion in neuroprotective clinical trials in 48 patients, and absence of follow-up in 94 patients. The ethics committee approved the protocol, and patients or their relatives gave informed consent.

The suspected cause of cerebral infarct was categorized following the Trial of Org 10172 in Acute Stroke Treatment criteria.[24,25] According to these classifications 134 were atherothrombotic infarcts, 143 cardioembolic, and 23 were undetermined. To simplify the analyses of our results, are studied undetermined infarcts together with the cardioembolic ones. TIA was defined as an acute focal loss of cerebral function with a presumed ischemic origin and with the focal neurological event resolving in less than 60 minutes.[26] We classified the TIA as ipsilateral or contralateral to the current infarction and, depending on its relationship in time, within 72 hours or beyond the 72 hours preceding the ischemic stroke. For the purposes of this study, and in agreement with our previous experience, we only considered relevant TIA for the ischemic preconditioning the ipsilateral TIA that occurred within the 72 hours preceding the cerebral infarction.[6,8] Patients with contralateral TIA or ipsilateral TIA beyond the prior 72 hours were included in the group of patients without TIA.

An experienced neurologist using the Canadian Stroke Scale quantified stroke severity at admission. The Canadian Stroke Scale ranges from 1.5 (maximum deficit) to 10 (absence of deficit).[27] Poor outcome was defined as death or Barthel Index score less than 85 at 3 months.

A computed tomography (CT) scan was performed on admission and repeated between days 4 and 7 of hospitalization. In the initial CT, the presence of early signs of ischemia was determined (hypodensity of the basal ganglia, effacement of the sulci, mass effect). Infarct volume was calculated in the second CT in accordance with the formula 0.5xaxbxc (a = greatest longitudinal diameter; b = transverse diameter; c = number of 10mm slices in which the infarct could be seen). Blood chemistry tests, 12-load electrocardiogram, chest radiography, and arterial supraaortic trunk examination also were performed in all patients. Only patients who had a systolic blood pressure 220mm Hg or higher or a diastolic blood pressure 120mm Hg or higher received antihypertensive drugs within the first 48 hours after admission. Treatment with insulin for hyperglycemia (blood glucose >160mg/dl) and with intravenous metamizol or paracetamol for hyperthermia (tympanic temperature >37°C) was initiated early after hospitalization. Subcutaneous low-dose heparin as prophylaxis against pulmonary thromboembolism and antiplatelet drugs were prescribed. Anticoagulants were given to patients with a major cardioembolic source but not as a treatment for early neurological deterioration.

Laboratory Tests

Blood samples were taken on admission in the emergency department in glass tubes containing potassium EDTA. Suspensions of plasma were centrifuged at 3,000*g* for 5 minutes and stored at −80°C. TNF-α and IL-6 were measured with commercially available quantitative sandwich enzyme-linked immunoabsorbent assay kits (Quantikine; R & D Systems, Minneapolis, MN) as previously described.[28] These measurements were made by technicians at a separate laboratory who were unaware of the clinical outcome and neuroimaging findings.

Statistical Analyses

We used the χ^2 test to compare proportions between the patients with TIA and patients without TIA. Continuous variables were expressed as mean ± SD and were compared using the Student's *t* test or analysis of variance test. CSS and Barthel Index were expressed as median (quartiles) and compared using the Mann–Whitney *u* test. The Kruskal–Wallis test was used to compare TNF-α concentrations between the two groups measured at five time intervals depending on the time from the onset of symptoms to blood sampling. The significance of the relationship between the volume of the infarct and the concentrations of TNF-α and of IL-6 on admission was calculated by using the Pearson's linear correlation coefficient.

Using the method described by Robert and colleagues,[29] we estimated the cutoff values for the plasma TNF-α and IL-6 concentrations with the highest sensitivity and specificity of being associated with prior ipsilateral TIA. This method is a probabilistic technique based on Bayes' rules that provides the maximum probability of a correct classification. With the cutoff values of TNF-α and IL-6, we created a new variable termed TNF-α/IL-6 index.

The importance of prior TIA (no = 0, yes = 1), TNF-α, IL-6, and the TNF-α/IL-6 index (negative = 0, positive = 1) on stroke outcome (good = 0, poor = 1) was analyzed in four models of logistic regression after adjusting for the variables obtained on admission which had influence on prognosis: age, temperature, serum glucose, CSS score, early signs of ischemia on CT (no = 0, yes = 1), and stroke subtype (atherothrombotic = 0, cardioembolic/undetermined = 1).

Results

Of the 283 patients studied, 38 had an ipsilateral TIA within the 72 hours preceding the cerebral infarction (13.4%). In 25 patients, it was the first TIA, whereas 13 patients had had previous transient ipsilateral events but beyond 72 hours. The length of the TIA was less than 15 minutes in 21 patients, 15 to 30 minutes in 12, and 30 to 60 minutes in 5 patients. Fifty-two pa-

tients classified as without relevant TIA for the ischemic preconditioning had prior history of TIA: 27 patients had contralateral TIA (only in 5 patients did they occur within 72 hours preceding infarction), and 25 had ipsilateral TIA but more than 72 hours beyond the current ischemic stroke. Among the overall excluded patients, the frequency of ipsilateral TIA within the 72 hours prior ischemic stroke was 11.7%, whereas in those diagnosed as having a lacunar infarction it was 3.6%.

The frequency of relevant TIA was significantly higher in those patients with atherothrombotic infarcts than in those with cardioembolic or undetermined infarcts (20.1 vs 7.4%, $p < 0.0001$). The baseline clinical and neuroradiological characteristics were similar in patients with and without relevant TIA, in both stroke subtypes (Table 1). The length of the TIA was similar in the two groups (atherothrombotic, 13.7 ± 11.2 minutes; cardioembolic/undetermined, 11.4 ± 14.5 minutes; $p = 0.183$). All patients with relevant TIA were receiving treatment with antiplatelet agents (6 aspirin, 16 clopidogrel) or anticoagulants (n = 16) at the time of the subsequent cerebral infarction. Among the patients without relevant TIA, 68 were receiving antithrombotic treatment (13 aspirin and 55 dicumarol) at the time of the ischemic stroke.

The frequency of poor outcome (death or Barthel Index score < 85) at 3 months, and the volume of the infarct, was significantly lower in patients with relevant TIA than in those without, both in the atherothrombotic and cardioembolic or undetermined infarcts (see Table 1). Interestingly, the better outcome observed in patients with an ipsilateral TIA within 72 hour preceding the cerebral infarction was not observed in those with an ipsilateral TIA more than 72 hours beyond the infarction (Barthel Index Score >85, 89.5% vs 36.5%, $p < 0.0001$).

In patients with previous TIA, plasma concentrations of TNF-α were significantly higher ($p < 0.0001$), whereas the concentrations of IL-6 were significantly lower ($p < 0.0001$), in comparison with the levels of patients without relevant TIA (Table 2). In the last group, TNF-α and IL-6 concentrations were similar in patients without any TIA, in those with ipsilateral TIA but more than 72 hours before the infarction, and in those with contralateral TIA, including patients in whom TIA occurred within 72 hours before infarction (Table 3). Patients with TIA showed increased concentrations of TNF-α at all the time intervals during the first 24 hours from the onset of symptoms ($p < 0.0001$; Fig 1). TNF-α concentrations in plasma had a positive correlation with infarct volume in patients without TIA ($r = 0.420$, $p < 0.0001$), whereas the correlation was negative in patients with TIA ($r = -0.756$, $p < 0.0001$). A similar effect was found between the concentration of IL-6 and the volume of the infarct, although it achieved only statistical significance in the group of patients without TIA ($r = 0.672$, $p < 0.0001$; Fig 2).

In the total series, we did not find a significant correlation between TNF-α and IL-6 concentrations in plasma ($r = 0.168$, $p = 0.05$; Fig 3A). However, TNF-α greater than 30pg/ml and IL-6 less than 30pg/ml discriminated between patients with previous TIA and those without (see Fig 3B). Consequently, we considered the TNF-α/IL-6 index as positive when TNF-α was greater than 30pg/ml and when IL-6 was less than 30pg/ml. Positive TNF-α/IL-6 index was

Table 1. Baseline and Outcome Clinical and Neuroradiological Variables

	Atherothrombotic Infarcts (n = 134)			Cardioembolic infarcts (n = 149)		
Variable	Without TIA (n = 107)	With TIA (n = 27)	*p*	Without TIA (n = 138)	With TIA (n = 11)	*p*
Age (yr)	67.9 ± 10.1	67.2 ± 5.9	0.450	65.1 ± 12.5	70.6 ± 1.9	0.227
History arterial hypertension (%)	57.0	48.1	0.270	15.9	0	0.161
History diabetes mellitus (%)	52.3	48.1	0.431	23.2	27.3	0.501
History atrial fibrillation (%)	8.4	3.7	0.363	38.4	36.4	0.583
Time from onset (hr)	8.7 ± 6.6	6.3 ± 3.5	0.498	6.9 ± 4.2	7.8 ± 4.8	0.746
Body temperature (°C)	37.2 ± 0.7	37.3 ± 0.5	0.772	37.1 ± 0.7	36.7 ± 0.4	0.153
Systolic BP (mm Hg)	149.9 ± 27.3	146.6 ± 31.5	0.156	139.5 ± 23.9	141.8 ± 16.6	0.746
Diastolic BP (mm Hg)	89.2 ± 18.7	83.7 ± 13.8	0.643	82.4 ± 14.8	86.4 ± 4.5	0.294
Serum glucose (mg/dl)	183.7 ± 74.5	168.2 ± 49.7	0.633	176.6 ± 63.5	166.4 ± 3.7	0.794
Fibrinogen (mg/dl)	506.1 ± 160.7	512.2 ± 123.9	0.716	474.3 ± 145.7	388.1 ± 111.8	0.054
CSS score (range)	6 (3–8.5)	7.5 (6–8.5)	0.106	5.5 (3.5–8)	7.5 (5–8.5)	0.069
Early signs on CT (%)	65.4	48.1	0.122	171.7	45.5	0.089
Poor outcome at 3 mo (%)	55.1	14.8	<0.001	55.8	0	<0.001
Ultimate infarct volume (ml)	45.2 ± 46.3	10.1 ± 4.1	<0.001	38.8 ± 36.3	11.8 ± 3.5	0.009

TIA = transient ischemic attack; BP = blood pressure; CSS = Canadian Stroke Scale; CT = computed tomography.

Table 2. Tumour Necrosis Factor and Interleukin-6 in Plasma on Admission

	Atherothrombotic Infarcts (n = 134)			Cardioembolic Infarcts (n = 149)		
Plasma Type	Without TIA (n = 107)	With TIA (n = 27)	*p*	Without TIA (n = 138)	With TIA (n = 11)	*p*
TNF-α (pg/ml)	11.4 ± 5.9	49.3 ± 10.3	<0.0001	14.3 ± 6.5	38.9 ± 7.9	<0.0001
IL-6 (pg/ml)	31.4 ± 14.5	12.7 ± 5.1	<0.0001	25.9 ± 18.9	3.5 ± 3.2	<0.0001

TIA = transient ischemic attack; TNF = tumor necrosis factor; IL = interleukin.

Table 3. Plasma Tumor Necrosis Factor and Interleukin-6 on Admission in Patients without Relevant TIA for Ischemic Preconditioning

Plasma Type	Patients without Any TIA (n = 193)	Patients with Ipsilateral TIA >72 hr (n = 25)	Patients with Contralateral TIA (n = 27)
TNF-α (pg/ml)	13.4 ± 6.7	11.5 ± 4.9	18.2 ± 3.1
IL-6 (pg/ml)	28.4 ± 17.3	27.9 ± 17.4	25.4 ± 17.4

TIA = transient ischemic attack; TNF = tumor necrosis factor; IL = interleukin.

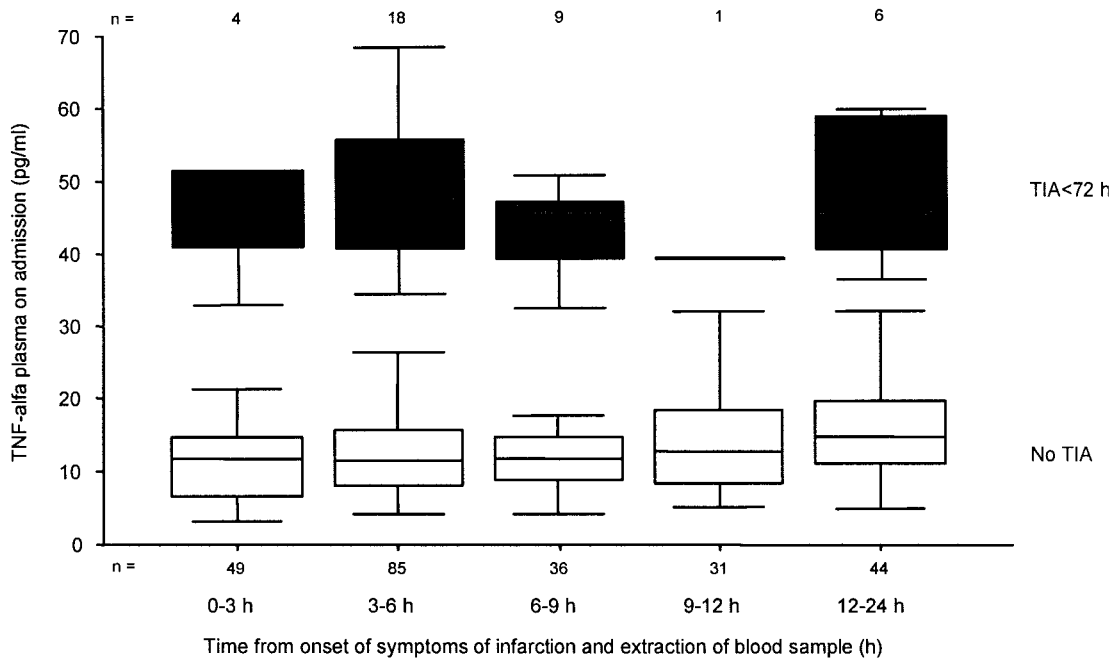

Fig 1. Plasma concentration of tumor necrosis factor (TNF)–α in patients with and without previous transient ischemic attack (TIA) in relation to the time from stroke onset to the extraction of the blood sample (Kruskal–Wallis, $p < 0.0001$*).*

found in 92.1% of patients with prior TIA and in 1.2% of patients without ($p < 0.0001$).

The logistic regression models demonstrated that the concentration of TNF-α in plasma ($p = 0.001$), but above all the positive TNF-α/IL-6 index ($p = 0.0003$) and a TIA within 72 hours preceding stroke ($p = 0.0001$), were associated with a better outcome at 3 months, whereas IL-6 in plasma was a predictor of poor outcome ($p = 0.030$; Table 4).

Discussion

These findings support the neuroprotective effects of TIA preceding ischemic stroke. TIA within the 72 hours before an ipsilateral cerebral infarct was associated with smaller infarct volume and better functional outcome. Importantly, this effect remained after adjusting for other prognostic variables and was independent of the stroke subtype. This study also gives some light to the molecular mechanisms that may be in-

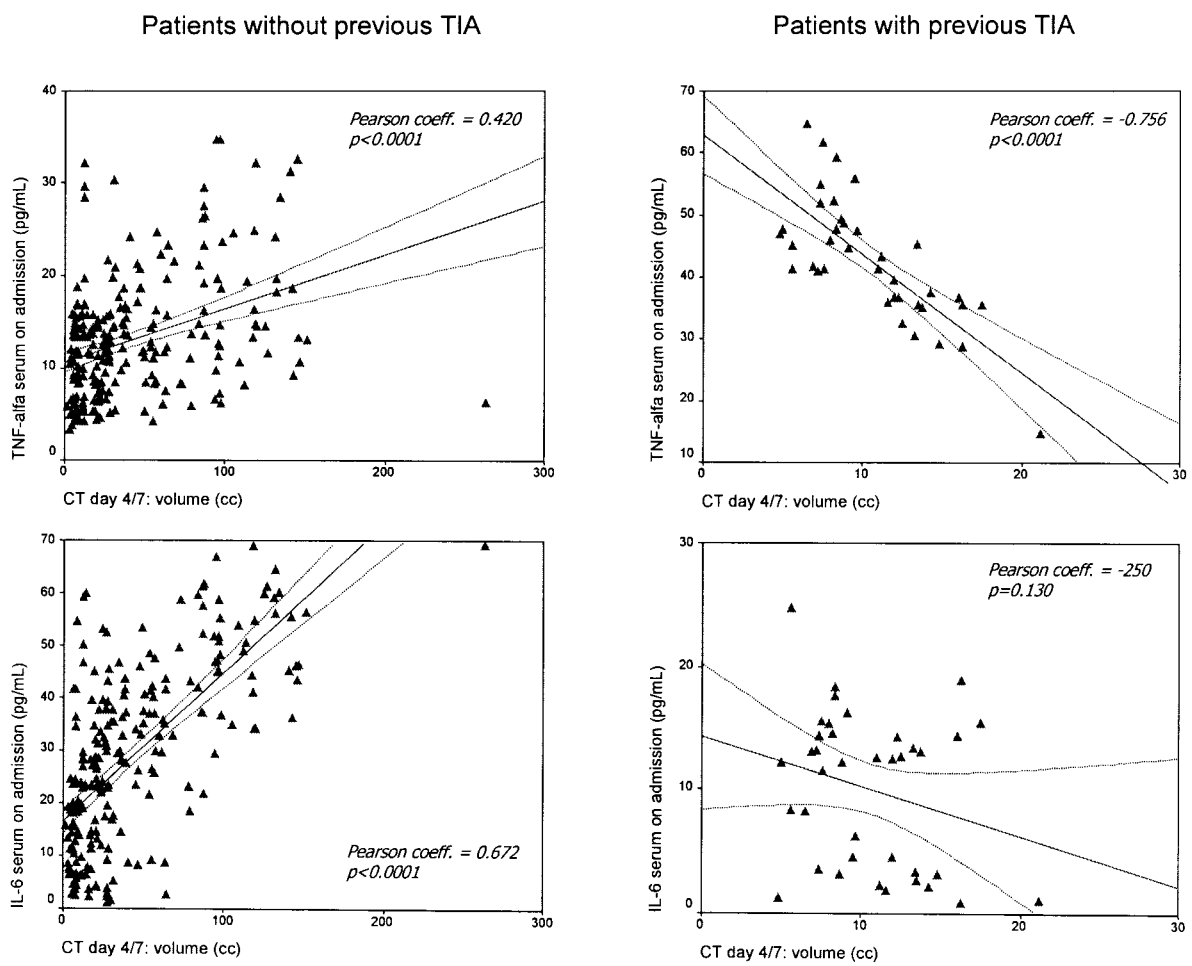

Fig 2. Scatterplots of the concentration of tumor necrosis factor (TNF)–α (top) and interleukin (IL)–6 (bottom) and the ultimate infarct volume measured on computed tomography performed between the fourth and seventh day, in patients without previous transient ischemic attack (TIA) (left) and in patients with prior TIA (right).

volved in the ischemic tolerance provided by a TIA. Likewise in experimental ischemic preconditioning, preceding TIA probably induced the expression of high concentrations of TNF-α and prevented the inflammatory response associated with the release of IL-6. The defined TNF-α/IL-6 index, a marker in blood of these two different molecular reactions, was the most powerful predictor of good outcome together with preceding TIA.

In experimental models, the effects of ischemic preconditioning depend on the duration of the ischemic events, the interval between events, and the number of events. The duration of the preconditioning must be greater than 5 minutes,[30] and the latency between the ischemic stimulus and the induction of the tolerance phenomenon must be greater than 24 hours.[31] Furthermore, the repetition of the ischemic events cancel their neuroprotective action.[32] Once induced, the phenomenon of ischemic tolerance is transient and disappears in a few days.[33,34] Some clinical findings have confirmed in humans these experimental results. In a case–control study of hemispheric cerebral infarctions, preceded or not by TIA, we found that only the ipsilateral TIA that occurred within the 72 hours before the cerebral infarction were associated with an improved outcome at 48 hours, at 7 days, and at 3 months.[6] Similarly, Moncayo and colleagues[7] demonstrated that TIA that occurred longer than a week before stroke did not change prognosis in comparison with that of patients without prior TIA. They also reported that prior TIAs with duration of between 10 and 20 minutes were associated with an improved outcome. In this work, we found a better outcome in patients with prior ipsilateral TIA within 72 hours than in those with a TIA more than 72 hours before the infarction. Therefore, according to the experimental and clinical evidence, we classified as relevant TIA for ischemic preconditioning those that preceded stroke within 72 hours in the ipsilateral hemisphere.

A further point of interest is that only short periods

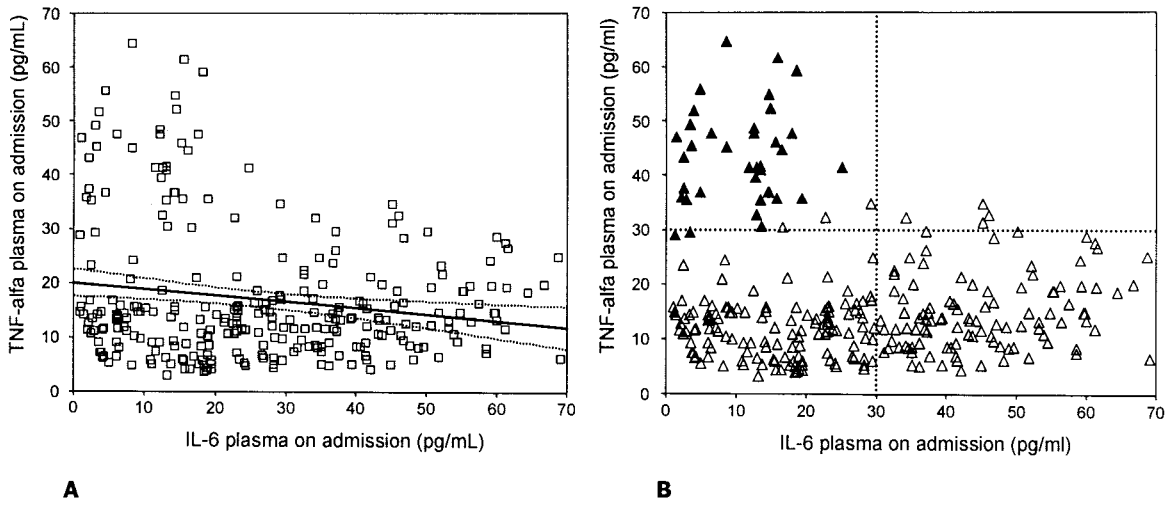

Fig 3. Scatterplot of the concentrations of tumor necrosis factor (TNF)–α and interleukin (IL)-6 in plasma. (A) Complete series (Pearson coefficient = 0.168; p = *0.05). (B) Patients with previous TIA* (filled triangles) *and patients without previous TIA* (unfilled triangles). *The 92.3% of the patients with previous transient ischemic attack (TIA) had concentrations of TNF-α >30pg/ml and of IL-6 < 30pg/ml.*

Table 4. Logistic Regression Models Using Dependent Variables: Death or Barthel Index Score <85

Variable	OR	CI (95%)	*p*
Model 1			
Age (y)	1.012	0.982–1.043	0.423
Temperature	3.127	1.794–5.448	0.0001
Glycemia	1.003	0.997–1.009	0.237
CSS	0.709	0.592–0.849	0.0001
Early signs of ischemia (1)	3.849	1.506–9.832	0.004
Diagnosis (1)	1.026	0.511–2.059	0.941
Prior TIA (1)	0.081	0.023–0.280	0.0001
Model 2			
Age	1.004	0.975–1.035	0.757
Temperature	2.330	1.337–4.023	0.002
Glycemia	1.002	0.996–1.008	0.394
CSS	0.710	0.597–0.843	0.0001
Early signs of ischemia (1)	3.367	1.399–8.102	0.006
Diagnosis (1)	0.716	0.370–1.368	0.322
IL-6	1.025	1.002–1.049	0.030
Model 3			
Age	1.008	0.978–1.039	0.585
Temperature	3.561	2.039–6.219	<0.0001
Glycemia	1.003	0.9987–1.009	0.205
CSS	0.698	0.588–0.828	<0.0001
Early signs of ischemia (1)	3.881	1.559–9.660	0.003
Diagnosis (1)	0.821	0.422–1.596	0.562
TNF-α	0.956	0.929–0.983	0.001
Model 4			
Age	1.010	0.980–1.041	0.498
Temperature	3.333	1.914–5.803	<0.0001
Glycemia	1.003	0.997–1.009	0.245
CSS	0.711	0.598–0.847	0.0001
Early signs of ischemia (1)	3.966	1.579–9.963	0.034
Diagnosis (1)	0.953	0.482–1.882	0.890
Tolerance biochemical factor (1)	0.138	0.047–0.406	0.0003

no = 0; yes = 1.

CSS = Canadian Stroke Scale; TIA = transient ischemic attack; IL = interleukin; TNF = tumor necrosis factor.

of ischemia not able to produce infarction confer ischemic tolerance in animal models.[1] In humans, large-scale studies have shown that most TIA resolve within 10 to 60 minutes, and that the likelihood that symptoms will resolve completely within 24 hours is less than 15% if symptoms last more than 1 hour.[35] These results accord with the high frequency on brain infarctions on CT, and particularly with the presence of consistent acute diffusion-weighted magnetic resonance imaging lesions, when TIA last more than 1 hour.[36] Furthermore, the mean duration of symptoms in TIA without ischemic lesions in diffusion-weighted magnetic resonance imaging is 0.96 hours, whereas it is 6.8 hours in patients with acute diffusion abnormalities.[37] Based on these findings, a new definition for TIA has been proposed: a TIA should have clinical symptoms typically lasting less than one hour, and without evidence of acute infarction.[38] However, neuroimaging would not be mandatory for this definition in other opinions.[39] Therefore, with the aim of selecting patients with short periods of focal cerebral ischemia that were unlikely to cause infarction, mimicking the experimental models of ischemic preconditioning, we decided to consider TIA those that were shorter than 1 hour.

A better knowledge of the mechanisms implicated in the phenomenon of ischemic tolerance may help in the development of new therapeutic strategies. Currently, these mechanisms are not well understood.[9] TNF-α is one of the proinflammatory cytokines that has been related to ischemic tolerance. TNF-α has pleiotropic activities that may lead to both detrimental[19,28,40,41] and neuroprotective[17–21] actions on brain cells depending on the experimental conditions. Interestingly, this dual response of TNF-α also has been observed in this clinical study. Although plasma concentrations of TNF-α were lower in patients without previous TIA, TNF-α levels correlated positively with infarct volume, whereas, in patients with prior TIA, the TNF-α had a clear neuroprotective effect, because the higher the TNF-α concentrations, the smaller the infarct volumes (see Fig 2). This effect was not related to the time interval from stroke onset to sampling, because the concentrations of TNF-α, both in patients with TIA and in those without, remained stable during the first 24 hours (see Fig 1).

To investigate the potential actions of TNF-α on the ischemic preconditioning, we analyzed the plasma concentrations of IL-6, a proinflammatory cytokine related to neuronal damage. IL-6 has shown neuroprotective[42] and neutral[43] and neurotoxic effects[44,45] in experimental models, the latter being associated with a pyretic action.[46] However, previous studies in humans have demonstrated an association between the inhibition of the inflammatory response, particularly of the IL-6 release, and the ischemic tolerance.[23] Consistent with these findings, we found lower concentrations of IL-6 in patients with prior TIA, whereas increased levels in patients without preceding TIA were associated with a worse prognosis and larger infarct volumes (see Fig 2; Table 4, model 2).

Our results provide support for the implication of TNF-α in ischemic tolerance. Preconditioning exposure to TNF initially was observed to confer cytoprotection as a potent inducer of manganese-superoxide dismutase.[47] More recently, the activation of transcription factors such as NF-κB has been implicated in ischemic tolerance,[14,16] although other mechanisms also can be involved. TNF-α reacting with its receptors leads to NF-κB activation and transactivation of genes that express proteins with proinflammatory, apoptotic, and cytotoxic actions in situations of cerebral ischemia,[16,19,48] as well as proteins that are antiinflammatory, antiapoptotic, and cytoprotective during ischemic preconditioning.[16,19,49,50] The precise mechanism by which activation of NF-κB leads to neurotoxic or neuroprotective effects is not well known,[19] although it is likely that different regulatory profiles of this transcription factor determine different outcomes. In this context, it has been shown that TNF-α induces NF-κB in a biphasic manner in different cells (reviewed in Ladner and colleagues[51]), including central nervous system cells.[52] It appears that TNF-α release profile determines the extent of each NF-κB phase, being the second phase in an important determinant toward a cytotoxic or cytoprotective fate.

This study has some limitations. First, although we cannot completely rule out a selection bias due to the exclusion of patients lost of follow-up, this bias is unlikely because the excluded patients showed similar clinical characteristics and frequency of TIA as the studied population. Second, the reported neuroprotective effects of TIA could be attributed to the differences in the natural history of TIA and the size of the emboli in atherothrombotic and cardioembolic cerebral infarctions, as has been suggested recently by Caplan.[53] Given the higher frequency of TIA in the former, and the larger sized emboli in the latter, a lower frequency of preceding TIAs could be associated with poorer outcome as a result of larger infarcts in cardioembolic strokes. We have not controlled for some baseline characteristics such as the severity of the occlusive lesions and the patency of the collateral circulation. However, the beneficial effects of prior TIA in this study were independent of the relevant prognostic variables such as age, initial stroke severity, body temperature, and serum glucose, and also of the stroke subtype. Third, the better outcome of patients with preceding TIA might be related to the prior administration of antithrombotic drugs. In our series, 100% of patients with prior TIA but 28% of those without TIA were taking antiplatelet agents or oral anticoagulants at the time of

the cerebral infarction. This difference was caused only by the different time frame between the prior TIA and the current stroke, because many patients withdrew treatment for secondary prevention with time. However, only aspirin treatment at stroke onset has been demonstrated to modify the natural course of cerebral infarct,[54] and the NF-κB is not inhibited by the low to medium doses of aspirin used in clinical routine.[55] Nevertheless, the number of patients taking aspirin was of little significance (only 6 patients in the TIA and 13 in the group without).

In conclusion, ipsilateral TIA within the 72 hours before the development of a hemispheric cerebral infarction are associated with an improved prognosis at 3 months. This ischemic tolerance also is associated with increased levels of TNF-α in the presence of reduced concentrations of IL-6 in plasma. Although this fact might implicate NF-κB as responsible, among other factors, for the phenomenon of ischemic tolerance in humans, similar to what occur in animals, future studies will be necessary to confirm this point. More exhaustive understanding of these mechanisms could open new therapeutic neuroprotective avenues of investigation.

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