Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuroscience Letters 398 (2006) 167–171
Neuroscience Letters
www.elsevier.com/locate/neulet

ACE gene polymorphisms influence t-PA-induced brain vessel reopening following ischemic stroke

Israel Fernández-Cadenas, Carlos Alberto Molina, José Álvarez-Sabín, Marc Ribó, Anna Penalba, Laura Ortega-Torres, Pilar Delgado, Manolo Quintana, Anna Rosell, Joan Montaner*

Neurovascular Research Laboratory and Neurovascular Unit, Departamento de Medicina Interna Universitat Autonoma de Barcelona, Edifici de Recerca, Vall d'Hebron Hospital, Pg Vall d'Hebron 119-129, 08035, Barcelona, Spain

Received 18 November 2005; accepted 29 December 2005

Abstract

Angiotensin converting enzyme (ACE) influences vessels tone and the coagulation/fibrinolysis system. The ACE gene I/D polymorphism has been linked with PAI-1 and fibrinogen levels and with Factors VII and X activities. Therefore, we aimed to test whether I/D polymorphism could be related to thrombolysis safety and efficacy. We studied strokes involving the middle cerebral artery (MCA) territory of patients who received t-PA <3 h of stroke onset. Blood samples were obtained before t-PA administration to measure fibrinogen, PAI-1, Factors VII and X. I/D polymorphism was determined by polymerase chain reaction and agarose electrophoresis. Recanalization rates were serially evaluated by Transcranial Doppler. Among 96 included patients the genotype frequency was: DD = 33.3%, ID = 57.3% and II = 9.4%. A strong association was found between DD homozygous and successful recanalization rates (DD = 69.2%, ID + II = 31.6%, $p = 0.002$ at 1 h; DD = 91.3%, ID + II = 51%, $p = 0.001$ at 6 h; DD = 100%, ID + II = 72.3%, $p = 0.003$ at 24 h post-t-PA administration). In fact, DD genotype was an independent predictor of recanalization (OR = 4.3 95% CI 1.35–13.49, $p = 0.013$). No relation was found between I/D polymorphism and symptomatic hemorrhagic complications ($p = 0.237$). No association between ACE genotypes and Factor VII or Factor X activities, neither with fibrinogen or PAI-1 levels was observed. DD homozygous is strongly associated with MCA recanalization following t-PA treatment. Mechanisms of benefit remain unknown since I/D polymorphism had similar FVII and X activities and PAI-1 and fibrinogen levels in our stroke population.

Keywords: Genes; ACE I/D polymorphism; Stroke; Recanalization; Hemorrhagic transformation

Tissue type plasminogen activator (t-PA) is the only approved drug for the treatment of acute ischemic stroke. Even though the great benefit of this treatment, between 6 and 15% of the stroke patients treated with t-PA suffer symptomatic hemorrhagic transformations (HT) [24] and as many as 40% of them do not recanalize or do it too late [10].

The reasons for interindividual differences in t-PA mediated clot lysis remain largely unknown. Angiotensin converting enzyme (ACE) is an enzyme that belongs to renin–angiotensin and kallikrein–kinin systems that plays a major role in vasoconstriction catalysing the transformation of angiotensin I to angiotensin II. An insertion/deletion (I/D) polymorphism in the intron 16 of the gene influences the levels of the circulating enzyme [15,22] and also of some endogenous fibrinolysis inhibitors [9,11].

In fact, the deletion allele (D) is associated with elevated levels of ACE and elevated levels of plasminogen activator inhibitor 1 (PAI-1) [9,11]. In contrast, recently it has been observed that the presence of the D allele in homozygous state determines low activities of coagulation Factors X and VII [12] and lower levels of fibrinogen [23] predisposing to an anticoagulant effect. Recent studies have shown that fibrinolysis related molecules may influence safety and efficacy after t-PA treatment in stroke [14].

Therefore, we aim to study the influence of I/D polymorphism of the ACE gene on Factor X and VII activities and fibrinogen and PAI-1 levels, and consequently determine its pro or antifibrinolytic effect and its impact on brain vessel recanalization and hemorrhagic transformations of t-PA treatment for stroke patients.

* Corresponding author. Tel.: +34 934894073; fax: +34 934894015.
E-mail address: 31862jmv@comb.es (J. Montaner).

0304-3940/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2005.12.079

Patients with an acute stroke admitted at the emergency room of a teaching hospital and enrolled consecutively were prospectively studied. Our target group consisted of those patients who had an acute ischemic stroke involving the vascular territory of the middle cerebral artery (MCA), and who fulfilled criteria for t-PA treatment. All patients underwent urgent carotid ultrasound and transcranial Doppler (TCD) examinations. Ninety-eight patients had a documented MCA occlusion on TCD and received t-PA in a standard 0.9-mg/kg dose (10% bolus, 90% continuous infusion during 1 h) <3 h after symptom onset. We excluded patients with a known inflammatory or malignant disease ($n = 2$). Finally, 96 patients were included in the analysis. All the included subjects were of European white ancestry (Mediterranean area).

A detailed history of vascular risk factors was obtained from each patient. To identify potential mechanism of cerebral infarction, a set of diagnostic tests was performed that included electrocardiogram, chest radiography, carotid ultrasonography, complete blood count and leukocyte differential and blood biochemistry in all patients; when indicated some patients also underwent special coagulation tests, transthoracic ecocardiography and Holter monitoring. With this information, and the neuroimaging data, previously defined etiologic subgroups were determined [1]. Stroke severity was assessed by using the National Institute of Health Stroke Scale (NIHSS). We defined neurological improvement as a decrease in the NIHSS stroke scale of 4 or more points and neurological deterioration as either death or an increase of 4 or more points at 48 h [2].

Neither intravenous heparin or antiplatelet agents were administered during the first 24 h. This study was approved by the Ethics Committee of the hospital and all patients or relatives gave informed consent.

A standard TCD examination was performed by experimented neurologists in the emergency room on admission before t-PA administration using I-channel 2-MHz equipment (TCD 100M, Spencer Technologies). A standard set of diagnostic criteria was applied to diagnose arterial occlusion. Proximal and distal MCA occlusions were defined according to the Thrombolysis in Brain Ischemia (TIBI) gradient system [5]. To asses recanalization, follow up recordings were performed at 1 h after t-PA administration and again at 6 and 24 h. In 13 patients the complete TCD follow-up was not available. Recanalization on TCD was diagnosed as partial when blunted or dampened signals appeared in a previously demonstrated absent or minimal flow. Complete recanalization on TCD was diagnosed if the end-diastolic flow velocity improved to normal or elevated values (normal or stenotic signals) [3]. No change in the abnormal waveforms indicated that no recanalization had occurred.

On admission, all patients underwent a CT within the first 3 h of stroke onset, and a second CT was repeated after 24–48 h (or earlier when rapid neurological deterioration occurred) to evaluate the presence of HT. CT scans were reviewed by a neuroradiologist with extensive experience in acute stroke that was blinded to clinical and laboratory details. Presence and type of HT were defined according to previously published criteria [7,13]. Symptomatic intracranial hemorrhage was defined as blood at any site in the brain on the CT scan and documentation of neurological deterioration.

DNA was extracted from whole blood by standard methods. A 490 base pairs in the intronic region of ACE where is located the I/D polymorphism was amplified by polymerase chain reaction (PCR) following previously described procedures [6]. The amplicons were analyzed on agarose gel electrophoresis (1.5%) and the homozygous DD were subjected to another insertion-specific amplification to prevent ID versus DD mistyping (10% of the homozygous DD are real ID, due to the preferential amplification of the shortest D allele and an inadequate amplification of the largest I allele) [20].

Peripheral blood was obtained from patients before t-PA administration, EDTA and citrated tubes were used to collect blood samples and plasma was obtained by standard methods. Plasma Factors VII and X activities were determined by a one-step assay using Factor VII deficient plasma, Factor X deficient plasma and PT-Fibrinogen HS plus (citrated tubes; Instrumentation Laboratory Company, Lexington, MA, USA) and carried out in a ST4 (Stago, France).

Plasma concentrations of PAI-1 were measured by enzyme-linked immunosorbent assay (ELISA) using the TintElize Biopool kit (Biopool, Sweden) according to manufacture's users guide.

Fibrinogen levels were measured by Von Claus-thrombin standard method (Grifols, Spain).

Descriptive and frequency statistical analyses were obtained and comparisons were made by use of the SPSS statistical package, version 12.0. Statistical significance for intergroup differences was assessed by the χ^2 or Fisher's exact test for categorical variables and to determine whether there was any significant difference in allelic or genotype frequencies between cases with or without MCA recanalization and with or without HT. Bonferroni correction was used to determine which genotype accounts for statistical significances. The presence of Hardy-Weinberg equilibrium for I/D polymorphism was examined by χ^2-test. *T*-test, Mann–Whitney *U*-test and one-way ANOVA were used to detect significant differences between age, NIHSS, Factor X, Factor VII, fibrinogen and PAI-1 with MCA recanalization and ACE polymorphism. To prevent overmodeling of the data, only recanalization and hemorrhagic transformation were studied in relation to patient's genotypes.

Logistic regression was achieved to determine the independent factors that could be predictors of recanalization of the vessel by the end of t-PA infusion.

A total of 96 patients (50.5% women, mean age 71.3 ± 10.5) with an ischemic stroke involving the MCA territory were included. Among them 50% of patients were hypertensive, 31.5% were dyslipemic, 20.7% had a history of diabetes mellitus and 39.1% had a documented atrial fibrillation. Median baseline NIHSS was 17 (range 4–28) and baseline TCD detected a proximal MCA occlusion in 60.9% and a distal occlusion in the remaining patients.

Homozygous DD was found in 32 patients (33.3%), heterozygous ID in 55 patients (57.3%) and homozygous II in 9 patients (9.4%) corresponding to a Hardy–Weinberg equilibrium ($p = 0.09$). No differences were observed among the three