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Predictive value of thrombospondin-1 for outcomes in patients with acute ischemic stroke

Jian-Bo Gao [a,*], Wei-Dong Tang [b], Hong-Xiang Wang [c], Yan Xu [c]

[a] Department of Emergency Medicine, Fuyang People's Hospital, 2–4 Guihua Road, Fuyang 311400, Zhejiang Province, China
[b] Department of Critical Care Medicine, Fuyang People's Hospital, 2–4 Guihua Road, Fuyang 311400, Zhejiang Province, China
[c] Department of Neurology, Fuyang People's Hospital, 2–4 Guihua Road, Fuyang 311400, Zhejiang Province, China

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ABSTRACT

Background: Thrombospondin-1 is a potent regulator of angiogenesis. The expression of cerebral thrombospondin-1 is promoted in a rat model of intracerebral hemorrhage. The current study was designed to investigate the change of plasma thrombospondin-1 concentrations and assess the prognostic value of plasma thrombospondin-1 concentrations for long-term mortality and functional outcome of ischemic stroke patients.
Methods: This study included 192 patients and 150 healthy controls. The plasma thrombospondin-1 concentrations were measured using enzyme-linked immunosorbent assay. An unfavorable outcome was defined as a modified Rankin Scale score >3. The relationships between plasma thrombospondin-1 concentrations and 6-month clinical outcomes were analyzed using multivariate analysis.
Results: Compared with healthy controls, plasma thrombospondin-1 concentrations were statistically significantly elevated in patients. Using multivariate analysis, thrombospondin-1 emerged as an independent predictor for 6-month mortality, 6-month unfavorable outcome and 6-month overall survival. Plasma thrombospondin-1 concentrations possessed high predictive values under receiver operating characteristic curve. Their predictive values were similar to those of National Institutes of Health Stroke Scale scores.
Conclusions: Plasma thrombospondin-1 concentrations are elevated obviously and are highly associated with long-term outcome of ischemic stroke.

1. Introduction

Thrombospondin-1 (TSP-1) is a 420–450-kDa homotrimeric multifunctional extracellular matrix protein, which was first isolated from human blood platelets as a thrombin-sensitive protein [1,2]. TSP-1 is also secreted from endothelial cells, fibroblasts, neutrophils, monocytes, and macrophages [3,4]. Interacting with multiple different cell surface receptors, proteins, and proteoglycans of specific domains, TSP-1 has various biological effects [5–10]. TSP-1 is a potent regulator of angiogenesis that functions to concurrently inhibit endothelial cell migration and the release of vascular endothelial growth factor from the extracellular matrix [11]. It has been demonstrated that the expression of cerebral TSP-1 is promoted in a rat model of intracerebral hemorrhage [12], suggesting that the striking increase of cerebral TSP-1 expression may reflect an attempt of antiangiogenic drives to impair endothelial cell growth and migration in brain tissue after intracerebral hemorrhage [13]. It has been noted that circulating TSP-1 concentrations are elevated in some illnesses including coronary artery disease, sickle cell disease and postoperative liver dysfunction [14–16]. However, at present there is a paucity of data available on the change of circulating TSP-1 concentrations after ischemic stroke.

2. Patients and methods

2.1. Study population

An observational, prospective study was carried out during the period of January 2010 to January 2013 in the Fuyang People's Hospital. The study was approved by the Institutional Review Boards at our hospital. Written informed consents from the subjects or from the family members were obtained. The study group consisted of consecutive patients with first-ever ischemic stroke initially evaluated at the emergency room and confirmed by brain magnetic resonance imaging. Exclusion criteria included concurrent renal or hepatic insufficiency, malignancy, recent infection, and surgery, or major trauma. The control group consisted of healthy individuals that were evaluated when they presented to our

Abbreviations: mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; TSP-1, Thrombospondin-1.

* Corresponding author.
E-mail address: FangLongZhu@yeah.net (J.-B. Gao).

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hospital and had blood collected as part of medical examination on January 2013.

2.2. Clinical assessment

The following variables were recorded for each patient: age, gender, body mass index, hypertension, hypercholesterolemia, diabetes mellitus, coronary heart disease, atrial fibrillation, peripheral arterial disease, smoking, National Institutes of Health Stroke Scale (NIHSS) score, stroke types (atherothrombotic, cardioembolic or lacunar), treatment during hospitalization, arterial blood pressure, and other laboratory results. An unfavorable outcome was defined as a modified Rankin Scale score >3 at 6 months and/or recurrent stroke and/or new cardiovascular events within 6 months. For follow-up, structured telephone interviews were performed by 1 doctor, blinded to clinical information and biomarker concentrations.

2.3. Determination of TSP-1 in plasma

Venous blood was drawn from patients on admission and from healthy controls at study entry. To minimize the confounding effect of platelet activation and degranulation ex vivo, possibly leading to artifactual elevation of TSP-1 concentrations, procession of blood samples was according to methods of Novelli et al. [15]. Plasma TSP-1 concentrations were in duplicates analyzed by enzyme-linked immunosorbent assay using commercial kits (R&D Systems, Minneapolis, MN) in accordance with the manufactures' instructions. Samples were all processed by the same laboratory technician using the same equipment and blinded to all clinical data.

2.4. Statistical analysis

Descriptive data were examined for all variables. All statistical analyses were performed using the Statistical Package for Social Science program (ver 19.0) and MedCalc for Windows (ver 9.6.4.0). Categorical variables were shown as numbers and percentages. Continuous variables were presented as mean ± SD. Comparisons were made by using the χ^2 or Fisher exact test for categorical data as well as unpaired Student's t test for continuous variables. The relationships between plasma TSP-1 concentrations and 6-month mortality and 6-month unfavorable outcome were assessed using a binary logistic regression analyses with calculated odds ratio (OR) and 95% confidence interval (CI). The predictive value was assessed using a receiver operating characteristic (ROC) curve analysis with calculated area under curve (AUC) and 95% CI. A combined logistic-regression model was configured to estimate the additive benefit of plasma TSP-1 concentrations to NIHSS scores. 6-month overall survival was estimated using the Kaplan–Meier method and the intergroup differences in survival time were tested using the log-rank test. Multivariate Cox's proportional hazard analysis was carried out to identify independent prognostic factors for overall survival with calculated hazard ratio (HR) and 95% CI. All significant parameters in the univariate analysis were entered into a multivariate model. A P of <0.05 was considered significant for all test.

3. Results

3.1. Study population characteristics

During the study period, 224 patients with first-ever ischemic stroke were initially evaluated at the emergency room and confirmed by brain magnetic resonance imaging. Of these, 23 patients were excluded according to exclusion criteria and 9 patients had a missing follow-up. Finally, 192 patients were included in the analysis. In this study, 150 healthy controls were recruited. There were no statistically significant differences in gender and age between patients and controls (both $P > 0.05$). Table 1 summarized the baseline demographic data, cardiovascular risk factors, and stroke characteristics of study participants. Compared with controls, patients had higher plasma TSP-1 concentrations (570.8 ± 225.5 ng/ml vs.145.9 ± 50.0 ng/ml, $P < 0.001$). Plasma TSP-1 concentrations were highly correlated with NIHSS scores ($r = 0.520$, $P < 0.001$).

3.2. Mortality prediction

Forty-three patients (22.4%) died in 6 months. Table 1 showed that non-survivors had higher plasma TSP-1 concentrations compared with survivors. A multivariate analysis identified NIHSS score (OR, 1.610; 95% CI, 1.322–1.960; $P < 0.001$) and plasma TSP-1 concentration (OR, 1.134; 95% CI, 1.075–2.101; $P < 0.001$) as the independent predictors for 6-month mortality of patients.

Fig. 1 showed that plasma TSP-1 concentrations predicted 6-month mortality of patients with high predictive performance based on the ROC curve. The predictive value of the plasma TSP-1 concentrations was thus similar to that of NIHSS scores (AUC, 0.904; 95% CI, 0.853–0.942; $P = 0.243$). In a combined logistic-regression model, TSP-1 improved the AUC of NIHSS scores to 0.938 (95% CI, 0.894–0.968), but the difference did not appear statistically significant ($P = 0.077$).

3.3. Unfavorable outcome prediction

Fourteen patients had recurrent stroke and 42 patients had new cardiovascular events. Eventually, one hundred and nine patients (56.8%) suffered from unfavorable outcome in 6 months. Just as shown in Table 2, plasma TSP-1 concentrations were statistically significantly higher in the patients with unfavorable outcome than favorable outcome. Using a multivariate analysis, NIHSS score (OR, 1.701; 95% CI, 1.428–2.016; $P < 0.001$) and plasma TSP-1 concentration (OR, 1.183; 95% CI, 1.081–2.146; $P < 0.001$) emerged as the independent predictors for a 6-month unfavorable outcome of patients.

Using a ROC curve analysis, plasma TSP-1 concentrations had high predictive performance for a 6-month unfavorable outcome of patients (Fig. 2). The predictive value of the plasma TSP-1 concentrations was similar to that of NIHSS scores (AUC, 0.877; 95% CI, 0.822–0.920; $P = 0.099$). In a combined logistic-regression model, TSP-1 numerically improved the AUC of NIHSS scores to 0.909 (95% CI, 0.858–0.930; $P = 0.092$).

3.4. Analysis of overall survival

During a 6-month follow-up, the mean overall survival time was 150.6 days (95% CI: 142.2–159.1) in all patients. Table 3 showed that overall survival during a 6-month follow-up was correlated with plasma TSP-1 concentrations and other variables. A multivariate analyses selected NIHSS score (HR, 1.409; 95% CI, 1.250–1.587; $P < 0.001$) and plasma TSP-1 concentration (HR, 1.078; 95% CI, 1.020–1.121; $P < 0.001$) as the independent predictors for overall survival during a 6-month follow-up.

In addition, plasma TSP-1 concentrations were bifurcated at mean value. High or low TSP-1 concentration indicated more or less than mean value. Thus, 92 patients had high TSP concentration. During a 6-month follow-up, the mean overall survival time was 123.6 days (95% CI: 108.4–138.8) in the patients with high TSP concentration; the mean overall survival time was 175.5 days (95% CI: 171.2–179.8) in the patients with low TSP concentration. Fig. 3 showed that overall survival time was statistically significantly shorter in patients with high TSP concentration than low TSP concentration.

4. Discussion

To our best knowledge, the current study for the first time determined the circulating TSP-1 concentrations and evaluated the relationships between plasma TSP-1 concentrations and long-term outcomes of ischemic stroke. Its main findings were that plasma TSP-1 concentrations were