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Changes in plasma thrombospondin-1 concentrations following acute intracerebral hemorrhage

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ABSTRACT

Background: Angiogenesis is a fundamental process for brain development and repair. Thrombospondin-1 is the first identified endogenous angiogenesis inhibitor. Its expression in rat brain is upregulated after intracerebral hemorrhage (ICH). We determined whether plasma thrombospondin-1 concentrations are associated with injury severity and prognosis in ICH patients.
Methods: This observational, prospective study recruited 110 patients and 110 age- and gender-matched healthy controls. Blood samples were collected from the patients at admission and from the healthy controls at study entry to measure plasma thrombospondin-1 concentrations. The endpoints included 1-week mortality, 6-month mortality, 6-month overall survival and 6-month unfavorable outcome (modified Rankin Scale score >2).
Results: Plasma thrombospondin-1 concentrations were markedly higher in patients than in healthy controls. Thrombospondin-1 was an independent predictive factor for all endpoints and plasma thrombospondin-1 concentrations were highly associated with injury severity reflected by hematoma volume and National Institutes of Health Stroke Scale score. Under receiver operating characteristic curves, plasma thrombospondin-1 concentrations had similar predictive values compared with hematoma volume and National Institutes of Health Stroke Scale score.
Conclusions: Increased plasma thrombospondin-1 concentrations following ICH are independently associated with injury severity and short-term and long-term clinical outcomes.

1. Introduction

Spontaneous intracerebral hemorrhage (ICH) is one of the most lethal types of stroke with the highest mortality and morbidity [1–3]. National Institutes of Health Stroke Scale (NIHSS) score and ICH volume are known to be associated independently with clinical outcomes after ICH [4–6]. There are endogenous mechanisms of brain self-repair after ICH, which include angiogenesis [7–9]. Angiogenesis is a fundamental process for brain development and repair, and it is finely tuned by a variety of pro-angiogenic and anti-angiogenic molecules [10,11]. Although most studies to date have focused on pro-angiogenic factors, anti-angiogenic factors have also gained increasing attention recently.

Thrombospondin-1 (TSP-1) is a 450 kDa trimeric glycoprotein, which is one of the members of the thrombospondin gene family [12–14]. It is produced and secreted into the circulation by some activated cells like platelets and endothelial cells [15,16]. TSP-1 is abundantly expressed following injury in the extracellular matrix and is identified as the first identified endogenous angiogenesis inhibitor [17,18]. The previous study has shown that ICH can induce angiogenesis in rat brain, accompanied by the upregulation of expression of TSP-1 [19]. It has been noted that circulating TSP-1 concentrations are increased in some illnesses including coronary artery disease, sickle cell disease and postoperative liver dysfunction [20–22]. We have recently demonstrated the close relationship between plasma TSP-1 concentrations and the prognosis of aneurysmal subarachnoid hemorrhage [23]. However, at present there is a paucity of data available on the change of circulating TSP-1 concentrations after ICH. Our aim was to assess plasma TSP-1 concentrations in ICH patients.

Abbreviations: ICH, intracerebral hemorrhage; NIHSS, National Institutes of Health Stroke Scale; TSP-1, thrombospondin-1.

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2. Materials and methods

2.1. Study population

This was an observational, prospective study carried out at the Jinhua People's Hospital between September 2010 and September 2013. We included patients with acute spontaneous basal ganglia hemorrhage admitted within the first 24 h from stroke. We excluded those patients with previous ischemic or hemorrhagic stroke, severe head trauma, use of antiplatelet or anticoagulant medication, presence of other prior systemic diseases including autoimmune diseases, uremia, liver cirrhosis, malignancy, and chronic heart or lung disease, recent infection (within a month), a surgical procedure or missing of follow-up.

Control group included age- and gender-matched healthy individuals. Control subjects were those who attended by our hospitals for healthy examination between September 2010 and September 2013. They showed normal blood and biochemical laboratory tests, namely differential blood count, hemoglobin concentration, total serum proteins, liver function tests, erythrocyte sedimentation rate, kidney function tests, and C-reactive proteins as well as were medically tested by a specialist and found free of any other medical illnesses.

The study was performed with the approval of the Human Investigation Committee at the Jinhua People's Hospital and written informed consent was obtained from the study subjects or their legal guardians. This study was registered in ClinicalTrials.gov (NCT02465671) on June 4, 2015 by Zhen-Yu Cheng.

2.2. Clinical and radiological evaluation

For each patient, the recorded information included sex, age, hypertension, diabetes mellitus, initial systolic blood pressure and diastolic blood pressure, and NIHSS score (assessed immediately after admission). Early neurological deterioration was defined as an increase of ≥4 points in the NIHSS score or death at 24 h from the onset of symptoms [24]. All patients underwent at least 2 cranial computerized tomography (CT) scans: an initial CT scan within 1 h of admission and follow-up CT scan at 24 h from the onset of symptoms. All CT scans were performed according to the neuroradiology department protocol and were reviewed by investigators blinded to the clinical information.

Table 1
The factors correlated with plasma thrombospondin-1 levels in patients with intracerebral hemorrhage.

Characteristics	r value	*P* value
Gender (m/f)	0.053	NS
Age (y)	0.106	0.272
Hypertension	0.080	NS
Diabetes mellitus	0.261	0.006
NIHSS score	0.584	<0.001
Hematoma volume (ml)	0.562	<0.001
Presence of intraventricular hemorrhage	0.283	0.003
Hemorrhage growth	0.195	0.041
Early neurological deterioration	0.211	0.027
Admission time (h)	0.013	NS
Plasma-sampling time (h)	0.111	NS
Systolic arterial pressure (mm Hg)	0.044	NS
Diastolic arterial pressure (mm Hg)	0.077	NS
Blood glucose level (mmol/l)	0.210	0.027
Plasma C-reactive protein level (mg/l)	0.222	0.020
Plasma D-dimer level (mg/l)	0.188	0.049
Blood white blood cell count ($\times 10^9$/l)	0.175	NS
Blood hemoglobin level (g/l)	0.177	NS
Blood platelet count ($\times 10^9$/l)	0.115	NS
Prothrombin time (s)	0.128	NS
Thrombin time (s)	0.137	NS
Partial thromboplastin time (s)	0.019	NS
Plasma fibrinogen level (g/l)	0.080	NS

Bivariate correlations were assessed by Spearman's or Pearson's correlation coefficient. NIHSS indicates National Institutes of Health Stroke Scale.

Hematoma volumes were measured according to the ABC/2 method, where A is the greatest hemorrhage diameter, B is the diameter perpendicular to A, and C is the number of slices multiplied by the slice thickness [25]. Hematoma growth was defined as hematoma enlargement >33% at 24 h [26].

2.3. Clinical endpoints

For follow-up, structure telephone interviews were performed by 1 doctor, blinded to clinical information and biomarker concentrations. Participants were followed up until death or completion of 6 months after stroke. An unfavorable outcome was defined as a modified Rankin Scale score >2 at 6 month. The end points were death within 1 week and 6 months and unfavorable outcome within 6 months after ICH.

2.4. Assay

Blood samples were collected from the patients at admission and from the healthy controls at study entry. Glycemia, hemoglobin, platelets, fibrinogen, C-reactive protein, D-dimer, prothrombin time, thrombin time, partial thromboplastin time and leukocytes were determined according to conventional methods. For the determination of TSP-1, blood samples were processed using methods of Novelli et al. [21]. Plasma TSP-1 concentrations were in duplicates analyzed by enzyme-linked immunosorbent assay using commercial kits 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.

2.5. Statistical analysis

Categorical variables were reported as numbers and percentages, and comparisons between groups were carried out using chi-square test or Fisher exact test. Continuous variables were presented as mean ± SD, and comparisons between groups were carried out using unpaired or paired Student *t* test. Correlations were analyzed by Spearman's correlation coefficient or Pearson's correlation coefficient and then followed by a multivariate linear regression.

Multivariate analysis was used to identify independent predictors of unfavorable prognoses after incorporating the variables that univariate analyses revealed to be associated with mortality and poor outcome. The relationships between plasma TSP-1 concentrations and clinical endpoints were assessed using binary logistic regression analyses with odds ratio (OR) and 95% confidence interval (CI).

The predictive values were assessed using receiver operating characteristic (ROC) curve analysis with estimated area under curve (AUC) and 95% CI. A combined logistic-regression model was conFig.d to estimate the additive benefit of plasma TSP-1 concentrations to NIHSS score and hematoma volume.

Overall survival was estimated using the Kaplan–Meier method and comparisons of survival time between groups were carried out using the log-rank test. Multivariate Cox's proportional hazard analysis was carried out to identify independent prognostic factors for overall survival with hazard ratio (HR) and 95% CI.

A *P* value of <0.05 was considered significant for all test. Statistical analyses were performed using the Statistical Package for the Social Sciences ver 19.0 and MedCalc ver 9.6.4.0.

3. Results

3.1. Study population characteristics

Initially, 150 ICH patients were assessed. We excluded forty patients with previous ischemic or hemorrhagic stroke (8 cases), severe head trauma (4 cases), use of antiplatelet or anticoagulant medication (5 cases), presence of other prior systemic diseases including autoimmune