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Clinical Study

Serum indoleamine 2,3-dioxygenase and kynurenine aminotransferase enzyme activity in patients with ischemic stroke

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ABSTRACT

We investigated the activation and pathophysiological roles of indoleamine 2,3-dioxygenase (IDO) and kynurenine aminotransferase (KAT) in patients with ischemic stroke. Patients were recruited from the acute stroke unit of a general hospital within 24 hours post-stroke. The immune transmission turbidity method was used to determine the concentration of serum high-sensitivity C-reactive protein (hsCRP), apolipoprotein A-1 and apolipoprotein B. The concentrations of triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein and non-esterified fatty acids were determined using an enzymatic method. Tryptophan (TRP), kynurenine (KYN) and kynurenine acid (KYNA) concentrations were determined by high performance liquid chromatography. The National Institutes of Health Stroke Scale (NIHSS) was used to assess the neurological deficits at admission and 3 weeks post-stroke. The IDO and KAT activity ratio were calculated by KYN/TRP and KYNA/KYN, respectively. The correlation between hsCRP and IDO, KAT and NIHSS score was also analyzed. A total of 81 patients with ischemic stroke and 35 normal controls were recruited. Lower TRP, KYNA, HDL and KAT activity ratio were found in the stroke group compared to the control group ($p < 0.05$). The levels of hsCRP and IDO activity ratio were much higher in the stroke group than the control group ($p < 0.01$). The IDO activity in patients with ischemic stroke showed a positive correlation with hsCRP (r = 0.425, $p = 0.027$). In addition, hsCRP and IDO levels were positively associated with the NIHSS score both at admission and 3 weeks post-stroke. These data suggest an inflammatory response characterized by up-regulated IDO activation in ischemic stroke, which might be closely relevant to its pathophysiology.

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1. Introduction

Stroke is a significant cause of morbidity and mortality. It is estimated that there are 4.5 million deaths a year from stroke worldwide and over 9 million stroke survivors, with the number of global deaths projected to rise to 6.5 million in 2015 and to 7.8 million in 2030 [1–3]. To date, stroke remains the leading cause of long-term disability. However, the pathological mechanism of central nervous system injury in post-stroke patients is not clear, although it may be related to the inflammatory response and oxidative stress. The systemic inflammatory response to acute ischemic stroke involves increases in several pro-inflammatory cytokines and C-reactive protein [4–6], and pro-inflammatory cytokines can activate the indoleamine 2,3-dioxygenase (IDO) enzyme, leading to the depletion of tryptophan (TRP) and the production of kynurenine (KYN), increasing the KYN/TRP ratio in peripheral blood. This ratio acts as a clinical measure of IDO activity [7].

Apart from being one of the 20 amino acids that constitute proteins, TRP is also a precursor for the synthesis of serotonin and L-KYN under certain physiological conditions. More than 95% of TRP is metabolized through the KYN pathway [8]. Most metabolites of the KYN pathway are neuroactive and have essential roles in the regulation of N-methyl-D-aspartate (NMDA) receptor function and free radical production. NMDA receptor-mediated excitotoxicity and excessive free radical production are involved in neurodegenerative disorders such as Huntington's disease, Parkinson's disease and Alzheimer's disease. Evidence suggests that KYN metabolism is altered in such diseases, and the possible therapeutic potential of the pharmacological modulation of this pathway is currently being investigated in preclinical studies [8]. To our knowledge few studies have been under taken of the activity of IDO and kynurenine aminotransferase (KAT). Therefore, we investigated serum IDO and KAT enzyme activity and explored the TRP pathway in patients with ischemic stroke.

2. Methods

2.1. Subjects

This study recruited 81 participants admitted to an acute care regional stroke center within 24 hours of ischemic stroke. The

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study was approved by the local Research Ethics Board, and all participants provided written informed consent. We also enrolled 35 healthy people as controls with normal heart, liver and kidney function. We excluded patients with diabetes mellitus, thyroid disease and a history of stroke.

2.2. Methods

All blood samples were collected within 24 hours after stroke, and the TRP, KYN and kynurenine acid (KYNA) levels were determined by high-performance liquid chromatography (HPLC).

2.3. Blood sampling and plasma analyses

After overnight fasting, 2 mL of venous blood was drawn from each participant. The collected samples were centrifuged at 2000 revolutions per minute (rpm) for 10 minutes within 30 minutes of collection and the serum samples were stored at −80 °C before analysis. Frozen serum was thawed at room temperature, and the thawed sample was deproteinized by adding an equal volume of 5% (v/v) perchloric acid. The acidified serum was vortexed, stood at room temperature for 10 minutes to precipitate the protein, and was then centrifuged for 10 minutes at 9000 rpm. Twenty μL of the supernatant was injected into the HPLC column for analysis. TRP, KYN and KYNA concentrations were determined by HPLC, as described elsewhere [9,10].

The level of TRP in serum was determined by comparison and superposition of peak retention time to external standards. The serum concentrations of KYNA and TRP were determined from their peak areas relative to an external standard according to the following equation: KYNA or TRP (nmol/L or μmol/L) = (the peak area of KYNA or TRP in serum)/(the peak area of KYNA or TRP in standard solution) × (the concentration of KYNA or TRP in standard solution) × 2. Data acquisition and processing were performed on the HPLC data processing system.

The immune transmission turbidity method was used to determine the concentration of serum high-sensitivity C-reactive protein (hsCRP), apolipoprotein A-1 (APOA1) and apolipoprotein B. The concentrations of triglyceride, cholesterol (CHO), high density lipoprotein (HDL), low density lipoprotein and non-esterified fatty acids (NEFA) were determined using an enzymatic method. Biochemical assays were performed by scientists blinded to all clinical information.

2.4. Neurologic function in stroke patients

The USA National Institutes of Health Stroke Scale (NIHSS) was used to assess the neurological deficits associated with stroke. The NIHSS is a maximum 42 point scale that assesses neurological deficit, and the greater the score the greater the severity of the stroke symptoms.

2.5. Statistical analysis

Continuous measures were summarized using mean ± standard deviation. TRP and KYN were determined by mass and converted to molar units. Their quotient was multiplied by 1000 to obtain the KYN/TRP activity ratio in units of μmol/mmol. The comparison between the normally distributed data was analyzed by *t*-test whereas the Mann–Whitney U test was applied for the comparison between abnormally distributed data. We also used Pearson correlations as appropriate.

All patient information was de-identified for statistical analyses using the Statistical Package for the Social Sciences software (version 17; SPSS, Chicago, IL, USA). $p < 0.05$ was set as the significance level.

3. Results

3.1. Subject characteristics

All 81 patients were diagnosed with ischemic stroke by head CT scan or MRI. They included 42 men and 39 women, with an average age of 64.1 ± 9.9 years (range 41–82 years). The stroke volume ranged from 19–21 mm. None of the 81 patients had diabetes and 55 were diagnosed with hypertension. None of the patients were on statins before the time of stroke.

The 35 control subjects were 20 men and 15 women, with an average age of 58.6 ± 17.5 (range 43–89 years). Nineteen of the control patients had hypertension and none of them used statins.

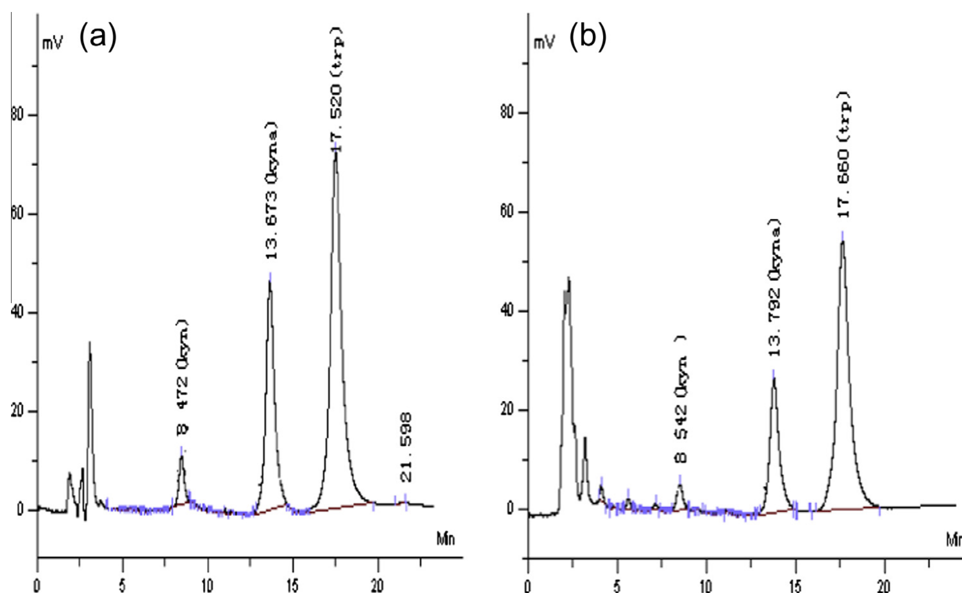

Fig. 1. The spectrum distribution figure of a (a) standard sample and (b) serum sample of (left to right) KYN, KYNA and TRP. KYN = kynurenine, KYNA = kynurenine acid, TRP = tryptophan.

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