Original Article
Changes in serum inflammatory cytokines, erythropoietin, vascular endothelial growth factor and orexins predict early depression after ischemic stroke

Yan Tan*, Bo Shang*, Yanxin Zhao, Xueyuan Liu

Department of Neurology, Tenth People's Hospital Affiliated to Tongji University, Shanghai 200000, China. *Equal contributors.

Abstract: This study aimed to investigate the serum level of monoamine neurotransmitters, inflammatory cytokines, leptin, EPO, VEGF, NGF and orexins in ischemic stroke patients with (n=38) and without (n=52) depression and explore the correlation between these parameters and post-stroke depression (PSD). When compared with non-PSD group, the VEGF, EPO, NE, 5-HT, DA and NGF reduced significantly (P < 0.05) and IL-6, TNFα, orexin-a and orexin-b increased significantly (P < 0.05) in PSD group. The leptin and IL-10 were comparable between two groups. The VEGF, EPO, NE, DA, NGF, IL-6, TNF-α, orexin-a, orexin-b, gender, MMSE score and HAMA score were risk factors of PSD in ischemic stroke patients. Logistic regression analysis showed PSD was independently related to HAMA score and orexin-b. These results indicate that in early PSD after initial ischemic stroke, inflammation is involved in the pathogenesis of PSD, and EPO, VEGF and factors able to promote neuroplasticity are protective on PSD. Orexin-b increase is closely related to PSD. The alteration in these parameters predicts the occurrence of PSD.

Keywords: Post-stroke depression, ischemic, orexin, inflammatory, erythropoietin

Introduction

Depression is one of common complications of stroke. It is a neuropsychiatric disorder characterized by low mood, loss of interest and pleasant sensation, lack of energy and fatigue as typical clinical symptoms. Post-stroke depression (PSD) has a high incidence at several weeks to months after stroke [1, 2]. About 1/3 of stroke patients survived will present clinical symptoms of depression [3]. PSD not only significantly affects the post-stroke rehabilitation and quality of life, but may increase the mortality [4]. Northern Manhattan Study (NOMAS) indicates that screening and interventions for post-stroke affective disorder early after acute stroke may significantly improve the prognosis [5]. However, PSD has insidious onset, its pathogenesis is still poorly understood, and there are no specific methods for the diagnosis and prediction of PSD. In this study, the correlation of blood biomarkers (such as monoamine transmitters, inflammatory cytokines, EPO, VEGF, LEPTIN and orexins) with PSD was evaluated in patients early after ischemic stroke.

Materials and methods

General data

Patients were consecutively recruited from the Department of Neurology of Shanghai 10th People's Hospital between June 2014 and February 2015. Informed consent was obtained from each patient before study and this study was approved by the Ethics Committee of the 10th People's Hospital.

Inclusion criteria: The diagnosis of PSD was made according to the diagnostic criteria for cerebrovascular diseases revised in the Forth National Cerebrovascular Disease Conference. The acute cerebral infarction was confirmed by cranial CT and MRI-DWI, and patients with initial ischemic stroke were admitted within 24 h after stroke.

Exclusion criteria: Following patients were excluded from this study: Patients received thrombolytic therapy after acute cerebral infarction; NIHSS score was ≥20 in patients with severe

Table 1. Clinical characteristic of patients in both groups

Clinical characteristics	PSD (n=38)	Non-PSD (n=52)
Gender		
Male	24 (63.2)*	19 (36.5)
Female	14 (36.8)	33 (63.5)
Age	70.17±10.786	68.66±9.607
Education level		
Junior School or lower	31 (81.6)	34 (65.4)
Senior School or lower	7 (18.4)	18 (34.6)
Lesion location		
Left	12 (31.6)	12 (23.1)
Right	13 (34.2)	25 (48.1)
Bilateral	5 (13.2)	5 (9.6)
Brainstem	8 (21.1)	10 (19.2)
Etiology		
Atherosclerosis	9 (23.7)	11 (21.2)
Cardiogenic factors	7 (18.4)	4 (7.7)
Lacunar infarction	10 (26.3)	16 (30.8)
Unknown causes	12 (31.6)	21 (40.4)
Risk factors of cerebrovascular diseases		
Hypertension	28 (73.7)	42 (80.8)
Diabetes	16 (42.1)	23 (44.2)
Atrial fibrillation	7 (18.4)	4 (7.7)
Smoking	4 (10.5)	6 (11.5)
Drinking	4 (10.5)	6 (11.5)
BMI≥28	0 (0.0)	4 (7.7)
Neurophysiological tests		
MMSE (score)	38 (9-30)**	44 (19-30)
HAMA (score)	21 (3-26)**	44 (0-2)
HAMD (score)	21 (3-31)**	44 (0-3)
NIHSS (score)	38 (0-12)	52 (0-7)

Notes: A total of 90 patients were included, including 38 in PSD group and 52 in non-PSD group. *P < 0.05, **P < 0.01 vs non-PSD group.

cerebral infarction; patients had hemorrhagic stroke; patients had recurrent cerebral infarction; patients had evident liver and/or kidney dysfunction; patients had severe heart failure or atrial fibrillation; patients had malignancies, severe systemic infection or a history of surgery or trauma within 4 weeks before stroke; patients had autoimmune diseases; patients had severe dementia, aphasia, dysarthria, deafness or other conditions that affected the Clinical Psychology Test; patients had a personal/familial history of mental illness; patients received pharmacotherapy for mental illness (including SSRI).

Diagnosis of PSD: The PSD was diagnosed at 7-10 days after stroke by 2 trained neurologists. If the patient was suspected with PSD, confirmed diagnosis was made according to the < Diagnostic and Statistical Manual of Mental Disorders: DSM-IV > criteria for mood disorder caused by physical illness (293.83). The 21-item Hamilton Depression Rating scale (HDRs21) was used to evaluate the severity of depression symptoms. The total score of < 8 indicates absence of depression, 8-17, mild depression, 18-24, moderate depression, > 24, severe depression. Results showed 38 patients were finally diagnosed with PSD including 24 males and 14 females.

Clinical characteristics: The clinical characteristics of recruited patients were collected and included 1) demographics; 2) risk for cardiovascular/cerebrovascular diseases: hypertension, diabetes mellitus and hyperlipidemia; 3) baseline characteristics related to stroke: body mass index and cognition.

Imaging examinations

SIEMENS sensation 64 splice helical CT and PHILIPS 3.0 T MR were employed for imaging examinations. CT scanning and MRI were performed within 24 h and 72 h after admission, respectively. According to the findings from CT/MRI, the nature and brain lesions locations were classified. The stroke was subtyped on the basis of Trial of ORG 10172 in Acute Stroke Treatment (TOAST). The evaluation of findings from imaging examinations was performed by the same radiologist who was blind to the study.

Sample collection and detections

Fasting venous blood was collected in dark. A fraction of blood was processed for routine blood test and detection of blood lipids, CRP, blood glucose, IL-6, IL-10 and TNF-α in the Laboratory Department of the 10th People's Hospital in Shanghai. The remaining blood was centrifuged at 3000 rpm/min for 5 min, and the plasma and blood cells were harvested independently and then stored at -80°C for use. Enzyme-linked immunosorbent assay (Shanghai Jierdun Biotech Co., Ltd) was employed for the detection of VEGF, EPO, leptin, orexin-a, orexin-b and monoamine neurotransmitters.

Table 2. Univariate logistic regression analysis of clinical factors related to PSD

Factors	β	SE	X^2 Walds	P	OR	95% CI
Gender	-0.191	0.443	6.073	0.014	0.336	0.141-0.800
MMSE (score)	-0.267	0.083	10.265	0.001	0.766	0.651-0.902
HAMD (score)	0.163	11.718	0.000	0.989	1.177	0.000-1109723522
HAMA (score)	2.158	0.76	8.064	0.005	8.657	1.952-38.403

Table 3. Multivariate logistic regression analysis of clinical factors related to PSD

Factor	β	SE	X^2 Walds	P	OR	95% CI
HAMA	2.134	0.849	6.32	0.012	8.446	1.600-44.572

Table 4. Clinical biochemical parameters in both groups

Parameters	PSD group (n=38)	Non-PSD group (n=52)
LDL	2.48±0.99	2.61±1.04
HDL	1.19±0.28	1.22±0.38
CRP (≥10)	25.48±19.63	16.90±4.63
PBG2h	8.54±3.65	7.85±3.22
HbA1C	5.81±1.88	5.88±1.81
VEGF	517.04±165.62**	670.88±145.73
EPO	19.18±6.16**	24.64±4.29
Leptin	3.05±0.69	3.01±0.73
NE	1023.24±262.84**	1265.14±246.12
5-HT	836.16±236.60**	1070.27±142.01
DA	49.30±13.95**	60.57±11.81
IL-6	4.56±1.06**	3.56±1.02
IL-10	146.74±38.59	146.63±44.10
TNF-α	222.90±44.90**	167.01±39.24
NGF	7.09±1.94*	8.08±1.66
IDE	26.42±6.50	26.45±5.80
Orexin-a	131.99±36.98*	115.96±38.14
Orexin-b	74.52±16.68**	64.16±16.13

Notes: A total of 90 patients were included, including 38 in PSD group and 52 in non-PSD group. *P < 0.05, **P < 0.01 vs non-PSD group.

Statistical analysis

Statistical analysis was performed with SPSS version 20.0. All the data were subjected to test of normal distribution with Shapiro-Wilk test. Continuous variables are expressed as mean ± standard deviation and compared between groups with T test or Mann-Whitney U test. Categorical variables are expressed as frequency or percentage and compared between groups with chi-square test. Correlation between normal distribution data was evaluated with Pearson correlation analysis. Correlation between variables with abnormal distribution was evaluated with Spearson correlation analysis. Univariate/multivariate logistic analysis was performed for the evaluation of risk factors. A value of *P* < 0.05 was considered statistically significant.

Results

On the basis of clinical observations and neuropsychological assessment, a total of 38 patients were diagnosed with PSD, and remaining 52 patients were included as non-PSD group.

Clinical characteristics of patients in both groups are shown in **Table 1**. When compared with non-PSD group, patients with PSD were men higher percentage (P < 0.05) and had significantly reduced MMSE score (P < 0.05) and significantly elevated HAMA score and HAMD score (P < 0.05).

Univariate logistic regression analysis (**Table 2**): Parameters with significant difference between two groups were included in univaivate logistic regression analysis. Results showed gender, MMSE score and HAMA score were found as risk factors of PSD in stroke patients.

Multivairate stepwise Logistic regression analysis (**Table 3**): The above 3 risk factors were subjected to multivairate stepwise Logistic regression, and results showed the occurrence of PSD was independently associated with HAMA score in stroke patients.

Risk factors of cerebrovascular diseases in both groups (**Table 4**): When compared with non-PSD group, the VEGF, EPO, NE, 5-HT, DA and NGF reduced significantly, but IL-6, TNF-α, orexin-a and orexin-b increased dramatically in PSD group (P < 0.05). There were no significant differences in leptin, IL-10 and IDE between two groups.

Univariate analysis of serum parameters (**Table 5**): The above parameters with significant dif-

Table 5. Univariate logistic regression analysis of blood risk factors of cerebrovascular diseases in PSD patients

Factors	β	SE	X^2 Walds	P	Exp (B)	95% CI
VEGF	-0.006	0.002	15.324	0.000	0.994	0.991-0.997
EPO	-0.192	0.047	16.441	0.000	0.825	0.752-0.906
NE	-0.004	0.001	14.307	0.000	0.996	0.994-0.998
5-HT	5.453	1.309	17.346	0.000	233.392	0.991-0.997
DA	-0.066	0.018	13.002	0.000	0.936	0.903-0.970
NGF	-0.307	0.124	6.117	0.013	0.736	0.577-0.938
IL-6	0.874	0.226	14.893	0.000	2.396	1.537-3.734
TNF-α	0.028	0.006	21.883	0.000	1.029	1.016-1.041
Orexin-a	0.011	0.006	3.783	0.052	1.011	1.000-1.023
Orexin-b	0.038	0.014	7.697	0.006	1.039	1.011-1.067

Table 6. Multivariate logistic regression analysis of blood risk factors of cerebrovascular diseases in PSD patients

Factors	B	SE	X^2 Walds	P	Exp (B)	95% CI
VEGF	-0.003	0.007	0.158	0.691	0.997	0.984-1.011
EPO	-0.331	0.219	2.273	0.132	0.719	0.468-1.104
NE	-0.004	0.003	1.548	0.213	0.996	0.989-1.002
DA	-0.099	0.09	1.223	0.269	0.905	0.759-1.080
NGF	0.337	0.513	0.432	0.511	1.401	0.512-3.830
IL6	1.544	0.849	3.309	0.069	4.683	0.887-24.711
TNFα	0.026	0.016	2.541	0.111	1.026	0.994-1.059
Orexina	0.025	0.026	0.918	0.338	1.026	0.974-1.080
Orexinb	0.098	0.048	4.256	0.039	1.103	1.005-1.212

ference between two groups were subjected to univariate logistic regression analysis. Results showed VEGF, EPO, NE, DA, NGF, IL-6, TNF-α, orexin-a and orexin-b were risk factors of PSD in stroke patients.

Multivariate stepwise Logistic regression analysis of serum parameters (**Table 6**): The above 9 parameters were included in multivariate stepwise Logistic regression analysis. Results showed orexin-b was independently related to PSD in stroke patients.

Discussion

This study indicated that the serum pro-inflammatory cytokines, orexin-a and orexin-b increased significantly, and monoamine transmitters, EPO and VEGF reduced dramatically in PSD patients, but the leptin, IDE and IL-10 were comparable between PSD patients and non-PSD patients.

Relevant studies have shown that increase in inflammatory cytokines can be observed at early stage of stroke, especially at 1 week after stroke, and thereafter remains for about 3 months or longer [6-8]. There is evidence showing that depression is related to persistent inflammation at a low level [9]. IL-6 and TNF-α are found as major cytokines involved in the pathogenesis of PSD [8] and even meta-analysis indicates that only IL-6 and TNF-α increased significantly in depression patients as compared to controls, and other cytokines remained unchanged (such as IL-1β, IL-4, IL-6, IL-8 and IL-10) [10]. However, the correlation between these inflammatory cytokines and PSD is still unclear. Our results showed pro-inflammatory cytokines and TNF-α increased significantly in patients with PSD, but IL-10 was similar between two groups. This suggests that inflammatory cytokines are involved in the pathogenesis of PSD at early stage, which was consistent with findings from the study of Yang et al. Yang et al investigated the serum cytokines in ischemic stroke patients who were admitted within 24 h after stroke. Their results showed the increased serum IL-18 on day 7 could predict the depression at acute phase and 6 months after stroke, but serum IL-6 and TNF-α were comparable between PSD patients and controls [11].

Leptin may affect the transduction of mono-amine transmitters and the regulation of HPA axis, which are involved in the development of depression [12]. A study was conducted to investigate the serum leptin in acute phase and 1 month after ischemic stroke in patients with initial ischemic stroke, and results indicated these serum leptin was higher in depression patients and serum leptin higher than 20.7 ng/ml was independently related to PSD [13]. In our study, results failed to show the relationship between leptin and PSD. The small sample size might be a reason for this discrepancy. In addition, the type of depression (presence of concomitant risk factors of cardiovascular diseases) may also affect the detection of serum leptin [14, 15].

EPO has a wide prospective in the treatment of depression, especially depression characterized by cognition impairment and may provide a new direction for the treatment of refractory decompression. EPO has neurotrophic and neuroprotective activities and plays important roles in the nerve development and cognition improvement [16, 17]. Moskowiak et al systemically reviewed the animal experiments and clinical studies on the treatment of depression and its relevant cognition impairment with EPO. Their results showed 4 animal experiments and 7 clinical trials suggested EPO was able to improve the hippocampus dependent memory impairment and exert anti-depression effect. They also proposed that the above effects of EPO were more likely to be mediated by the regulation of Neurobiological behaviors, but not the up-regulation of red blood cells [18].

Studies have confirmed the VEGF has the neurotrophic and neuroprotective activities and is related to the neurogenesis in the hippocampus. In addition, it affects the hippocampus dependent processes (such as memory and learning) and plays important roles in the synaptic transduction and stress [19, 20]. Currently, the results about the VEGF level of peripheral blood are conflicting in depression patients. Meta-analysis showed peripheral blood VEGF is higher in depression patients and may a promising biomarker of depression [19]. Kotan et al failed identified the significant difference in serum VEGF between healthy controls and patients with initial depression or recurrent depression [21]. However, Jennifer et al found VEGF reduced significantly in depression animals and the SSRIs were able to increase the VEGF expression [22]. The discrepancy in available findings on VEGF is related to sample size and methods used for measurement and analysis. In addition, our research was carried out under the background of early ischemic stroke and the pathophysiological mechanism is much more complicated. The VEGF as a compensatory pathophysiological mechanism to attenuate depression related neural deterioration might not occur under this condition, which may also cause the discrepancy of findings between studies. This study also indicates that the relationship between VEGF level and extent of VEGF related signaling pathway activation in the brain is still unclear. That is, detection of peripheral VEGF can not reflect the actual condition of central nervous system [19].

Orexins include orexin-a and orexin-b. Orexins are mainly synthesized and secreted by the neurons in the lateral hypothalamic (LH) area, dorsal medial hypothalamic (DMH) and perifornical area (PFA). They are involved in multiple neural activities via extensive neural projections. Orexins were initially regarded as being able to stimulate food intake and cause insomnia and energy consumption [23, 24]. In recent years, studies also indicate that orexins are closely related to the pathogenesis of Parkinson's Disease [25], cognition impairment [26] and depression [27].

Orexins are involved in the pathogenesis of depression via several mechanisms. For example, it may increase BDNF release and then BDNF may regulate the neuronal plasticity to inhibit the occurrence of depression. Study has shown the serum BDNF reduces significantly in depression patients [28]. In addition, orexins may also increase the intracellular calcium in dopaminergic neurons, leading to dopaminergic disorder and anhedonia [29]. Orexins also have anti-inflammatory activity while inflammation plays an important role in the pathogenesis of depression [30].

Our results showed serum orexins increased significantly in early stage of depression in patients with ischemic stroke, which was different from the findings in studies on the relationship between orexins and depression. Rotter et al found serum orexin-a reduced in depression patients, which was negatively related to the severity of depression [31]. Palhagen et al found orexin-a in the cerebral spinal fluid was higher in depression patients than in Parkinson's disease patients with or without depression, and anti-depression therapy reduced orexin-a [32]. Of note, the backgrounds in these studies were different from ours, the pathophysiology of PSD is more complex, and the alterations of inflammatory factors, neural factors, glucose and leptin may also affect the activity of orexin system [33]. Orexin positive neurons are widely distributed in the nervous system, including hypothalamus, brainstem, limbic system, cortex, spinal cord and vagus nerve [34], and thus the damage to these tissues may cause the release of orexin, affecting our results.

In addition, increase in orexins, especially orexin A, are frequently found in the cerebral spinal cord of depression patients. Orexin-a is fat-soluble and may diffuse through the blood brain barrier via simple diffusion, but orexin-b may be rapidly degraded in blood. Thus, it is difficult to detect orexin-b in the blood [23]. Although our results showed orexin-b was independently related to PSD, the conclusion should be elucidated cautiously.

Brundin et al found orexin-a in the cerebral spinal cord reduced significantly in depression patients as compared to those with emotion dysregulation, but increased significantly 6 and 12 months later [35], which indicates that conclusion should be further confirmed if detection of orexins are done at a specific time point and the symptoms of depression are not classified.

Studies have confirmed the interaction between orexins and Monoamine transmitters, but their roles in depression are poorly understood. Previous study has revealed that norepinephrine [36, 37] and dopamine [38] are able to inhibit orexin positive neurons. In addition, this effect is also noted in adrenaline, and moreover orexin positive neurons also express α1/2 adrenergic receptors. Catecholamines may inhibit orexin positive neurons via activating the α2 aadrenergic receptors mediated GIRK channel, which then regulate glutamatergic and GABAergic neurotransmitter to indirectly affect the activity of orexin positive neurons [39]. In addition, there is evidence showing that orexin-a in the cerebral spinal cord is higher in depression patients, but reduced after sertraline treatment for 5 weeks [40]. Thus, some investigators speculate that it is possible that depression reduces the monoamine transmitter in the center and periphery, which increases orexin as a compensation.

Conclusion

In early PSD of patients with initial ischemic stroke, inflammation is involved in the pathogenesis of PSD. Neuroplasticity related cytokines such as EPO and VEGF may be neuroprotective effect in PSD. Orexin-b increase is closely related to the occurrence of PSD. These changes may predict the occurrence of PSD in patients with initial ischemic stroke.

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Disclosure of conflict of interest

None.

Address correspondence to: Yanxin Zhao and Xueyuan Liu, Department of Neurology, Tenth People's Hospital Affiliated to Tongji University, Shanghai 200000, China. E-mail: zhao_yanxin@126.com (YXZ); liuxy@tongji.edu.cn (XYL)

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