European Journal of Inflammation

Letter to the Editor

European Journal of Inflammation
Volume 16: 1–5

DOI: 10.1177/2058739218819674
journals.sagepub.com/home/eji
SAGE

Preliminary findings on the expression of plasma CD63, CD62P, and PAI 1 in patients with acute cerebral infarction

Donghua Xu, Zhanyun Ren, Junfeng Shi, Guoxiang Wang, Yaxin Qin, Jin Zhang, Chengfang Chen and Wuzhuang Tang

Abstract

To investigate the expression and clinical significance of lysosomal granule glycoprotein 63 (CD63), P-selectin (CD62P) and endothelial cell plasminogen activator inhibitor (PAI-1) in patients with acute cerebral infarction. A total of 106 patients with acute cerebral infarction (ACI) admitted to our hospital from January to July in 2017 were selected as the patient group; 80 healthy subjects for physical examination in our hospital were selected as the control group. The expression levels of serum CD63, CD62P, and PAI-1 of the subjects were detected. The levels of CD63, CD62P, and PAI-1 in the serum of the patient group were significantly higher than those in the control group. There was a positive correlation between serum CD63 and CD62P ($r = 0.672$, $P < 0.05$) in the patient group. There was a positive correlation between serum CD63 and PAI-1 ($r = 0.643$, $P < 0.05$) in the patient group. There was also a positive correlation between serum CD62P and PAI-1 ($r = 0.601$, $P < 0.05$) in the patient group. Moreover, in other subtypes of cerebral infarction, the expression of CD63, CD62P, and PAI-1 was significantly higher than that of lacunar infarction. CD63, CD62P, and PAI-1 are highly expressed in peripheral blood mononuclear cells (PBMC) and serum of patients with ACI, which may be closely related to the occurrence and development of patients with ACI. These indices may be used as indicators of clinical diagnosis and prognosis in patients with ACI.

Keywords

acute cerebral infarction, CD63, CD62P, PAI-1, plasma

Date received: 28 April 2018; accepted: 26 November 2018

Acute cerebral infarction (ACI) is a relatively common stroke disease, the incidence of which is high and the morbidity is also high, seriously affecting people's health. In the event of ACI, it not only can cause limb dysfunction but also can cause cognitive impairment, both of which can cause the decline in quality of life of patients.[1] To this end, great importance and timely symptomatic treatment should be given to the disease, so as to avoid a great threat to the life safety of patients. Studies have confirmed that[2] in the pathophysiology of ACI, early endothelial cell injury, increased expression of inflammatory factors, and changes in their fibrinolytic activity are all important risk factors for ischemic stroke. Therefore, further study of its mechanism may play a positive role on the treatment and prevention of cerebral ischemic damage. Lysosomal granule glycoprotein 63 (CD63), P-selectin (CD62P), and endothelial cell plasminogen activator inhibitor (PAI-1) are more common molecular markers.[3] At present, the studies on

Department of Neurology, The People's Hospital of Yixing City, Yixing, China

Corresponding author:
Wuzhuang Tang, Department of Neurology, The People's Hospital of Yixing City, Yixing 214200, Jiangsu, China.
Email: xiezhong5285@163.com

plasma expression of CD63, CD62P, PAI-1, and other molecular markers in ACI are relatively small. The aim of this study was to investigate the expression and clinical significance of CD63, CD62P, and PAI-1 in patients with ACI, so as to reveal the pathogenesis of ACI and provide valuable reference for clinical diagnosis and prognosis of patients with ACI.

Materials and methods

General information

A total of 106 patients with ACI admitted to our hospital from January to July in 2017 were selected as the patient group. We further divided the patients with cerebral infarction into lacunar infarction (n = 18, infarct size <1.5 cm), small infarction (n = 22, infarct size: 1.5–3 cm), middle infarction (n = 31, infarct size: 3–5 cm), large infarction (n = 26, infarct size >5 cm), and multiple infarction (n = 9, multiple infarct lesions) according to computed tomography (CT) examination. Inclusion criteria are as follows: (1) patients in line with the diagnostic criteria for ACI in Western medicine; (2) patients with the relevant responsible lesions confirmed by head CT or magnetic resonance imaging (MRI) examination; and (3) patients with informed consent to accept the study. Exclusion criteria are as follows: (1) patients combined with other important organ diseases such as heart, liver, and kidney; (2) patients with incomplete clinical data; (3) patients with transient ischemic attack; (4) psychosis; and (5) patients with malignant tumors. There were 56 males and 50 females, aged 47–78 years (mean: 67.4 ± 2.1 years).

At the same time, 80 healthy people in our hospital were selected as the control group, including 44 males and 36 females, aged 46–77 years, mean (67.1 ± 1.8) years. There was no significant difference in sex and age between the two groups ($P > 0.05$).

Methods

Major reagents

Major reagents used are human CD63 enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Keshun Biotechnology Co., Ltd.), human CD62P ELISA kit (Shanghai Rongweida Industrial Co., Ltd.), human PAI-1 ELISA kit (Shanghai Hexu Biotechnology Co., Ltd.), DNA extraction reagent (Beijing Noble Ryder Technology Co., Ltd.), TRIzol Reagent (Beijing Biosn Technology Co., Ltd.), reverse transcription kit (Shenzhen Baoankang Biological Co., Ltd.), and DNA polymerase (Shanghai Yeasen Biotech Co., Ltd.).

Serum collection and treatment

In the early morning, 5 mL of fasting venous blood were collected from every subject, and after heparin anticoagulation, they were placed at low temperature for cryopreservation.

Laboratory inspection index detection

The expression levels of CD63, CD62P, and PAI-1 were detected by ELISA.

Detection of expressions of CD63 messenger RNA, CD62P mRNA, and PAI-1 mRNA in peripheral blood mononuclear cells

The appropriate amount of lymphocyte separation solution was added to the serum of the subjects, the mononuclear cells were separated by low-temperature centrifugation, and peripheral blood mononuclear cells (PBMC) was obtained after washing with RPMI-1640. The total RNA was extracted using TRIzol reagent, and the reverse transcription reaction was carried out according to the instructions of human CD63 ELISA kit, human CD62P ELISA kit, and human PAI-1 ELISA kit. Then, 10 μL of amplification products were obtained and electrophoresed in 20 g/L agarose gel. Photos were taken under ultraviolet (UV) light, and the films were scanned by the scanning densitometer. The ratio of CD63 messenger RNA (mRNA), CD62P mRNA, PAI-1 mRNA, and β-actin band gray scales was calculated to indicate the expression level.

Statistical processing

The data were analyzed by SPSS 20.0 statistical software. The measurement data were indicated by $\bar{x} \pm s$, tested by χ^2, and inspected by paired sample t-test. The correlation analysis of serum CD63, CD62P, and PAI-1 in the patient group was performed through Pearson correlation analysis. $P < 0.05$ was statistically significant.

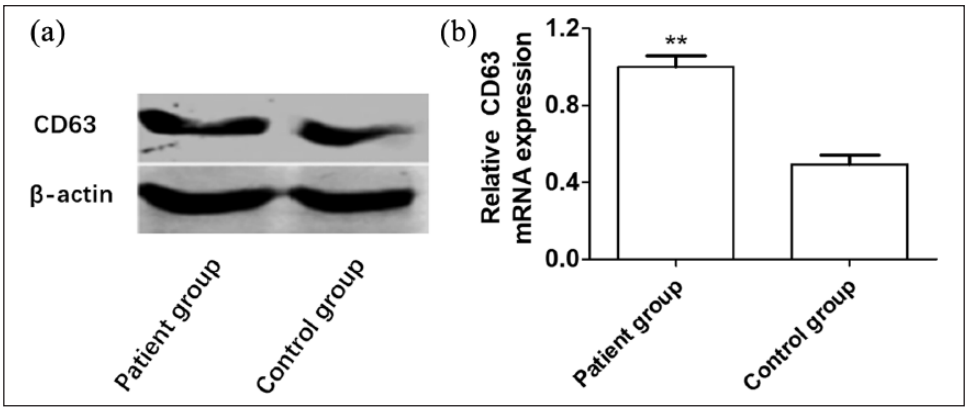

Figure 1. Comparison of the expression of CCR3 mRNA in PBMC of two groups: (a) CD63 electrophoregram and (b) semi-quantitative analysis (1: patient group and 2: control group. 1 vs 2, $^{**}P < 0.05$).

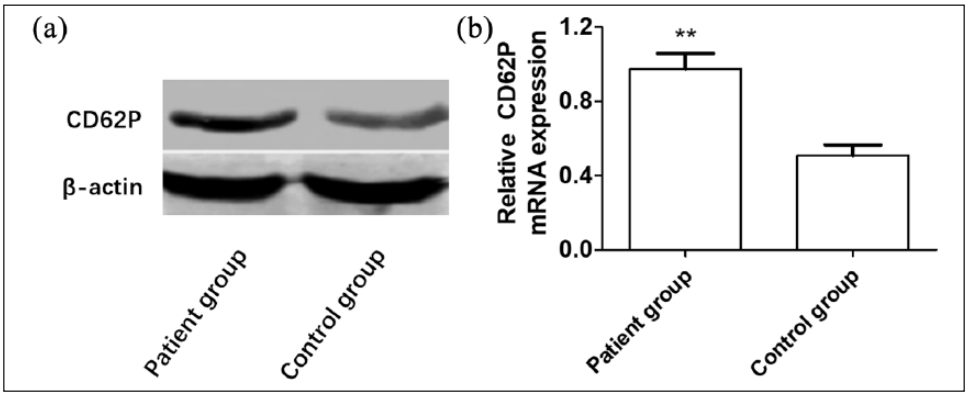

Figure 2. Comparison of CD62P mRNA expressions in PBMCs between two groups: (a) CD62P electrophoregram and (b) semi-quantitative analysis (1: patient group and 2: control group; 1 vs 2, $^{**}P < 0.05$).

Results

Comparison of CD63 mRNA expressions in PBMCs between two groups

The expression level of CD63 mRNA in the patient group was 0.964 ± 0.037, which was significantly higher than that in the control group (0.165 ± 0.017, $t = 12.394$, $P = 0.000$), as shown in Figure 1.

Comparison of CD62P mRNA expressions in PBMCs between two groups

The expression level of CD62P mRNA in the patient group was 0.878 ± 0.041, which was significantly higher than that in the control group (0.154 ± 0.025, $t = 27.885$, $P = 0.000$), as shown in Figure 2.

Comparison of PAI-1 mRNA expressions in PBMCs between two groups

The expression level of PAI-1 mRNA in the patient group was 0.742 ± 0.035, which was significantly higher than that in the control group (0.134 ± 0.012, $t = 29.093$, $P = 0.000$), as shown in Figure 3.

Comparison of CD63, CD62P, and PAI-1 contents in serum between two groups

The levels of CD63, CD62P, and PAI-1 in the serum of the patient group were significantly higher than those in the control group ($t = 8.325$, 8.004, 7.816, $P < 0.05$), as listed in Table 1.

Comparison of CD63, CD62P, and PAI-1 contents in serum among different subtypes of cerebral infarction

As shown in Figure 4, the expression level of CD63, CD62P, and PAI-1 mRNA in the lacunar infarcts patient group was significantly higher than that in the control group. Interestingly, in other subtypes of cerebral infarction, the expression of CD63, CD62P, and PAI-1 was significantly higher than that of lacunar infarction.

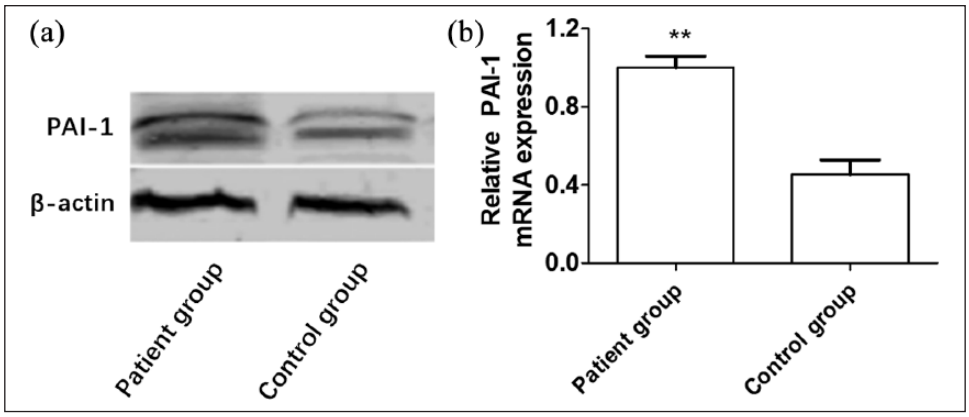

Figure 3. Comparison of PAI-1 mRNA expressions in PBMCs between two groups: (a) PAI-1 electrophoregram and (b) semi-quantitative analysis (1: patient group and 2: control group; 1 vs 2, $^{**}P < 0.05$).

Table 1. Comparison of CD63, CD62P, and PAI-1 contents in serum between two groups (ng/L).

Groups	n	CD63	CD62P	PAI-1
Patient group	106	795.2 ± 32.6	703.7 ± 26.5	674.3 ± 29.2
Control group	80	173.4 ± 17.8	151.6 ± 21.4	137.2 ± 15.1
t		29.732	29.336	30.224
P		0.000	0.000	0.000

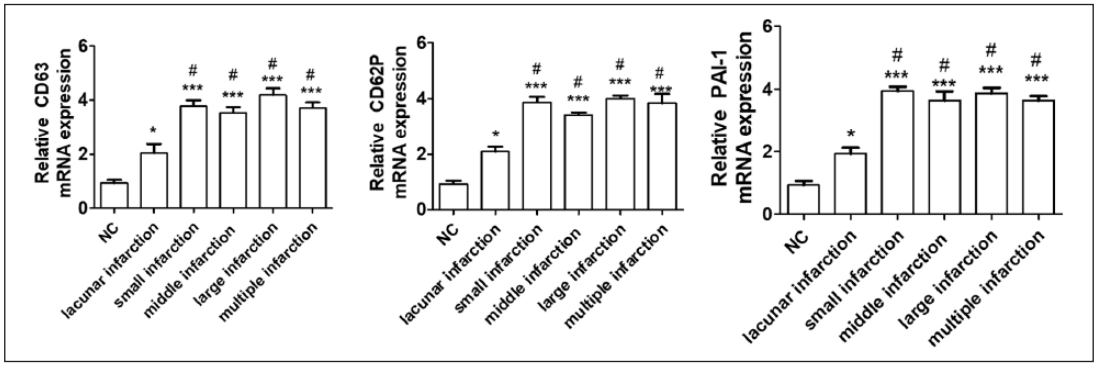

Figure 4. Comparison of CD63, CD62P, and PAI-1 mRNA expressions in PBMCs among five subtypes of cerebral infarction.

Correlation analysis

There was a positive correlation between serum CD63 and Crohn's disease activity index (CDAI) levels ($r=0.672$, $P<0.05$) in the patient group. There was a positive correlation between serum CD63 and PAI-1 ($r=0.643$, $P<0.05$) in the patient group. There was also a positive correlation between serum CD62P and PAI-1($r=0.601$, $P<0.05$) in the patient group.

Discussion

Cerebrovascular disease is the second cause of death in humans. The mortality of the disease within 1 year of the incidence can be as high as 21%–27%, and about 15%–30% of survivors will be left with life-long disability.[4] Cerebral infarction is a neurological dysfunction caused by brain blood supply block due to multiple factors. ACI is occlusion, cerebral insufficiency, and brain dysfunction caused by cerebral vascular sclerosis.[5]

Related studies[6,7] confirm that platelet activation plays an important role in the pathogenesis of ACI. CD63, CD62p, and PAI-1 are glycoprotein expressed on the surface of platelet membrane when platelet is activated. The expression of CD62p on the platelet membrane or in the percentage of its positive platelets is considered to be a specific marker of platelet activation. Our results showed that the levels of CD63, CD62P, and PAI-1

in control group were significantly lower than that in the patient group, which may be the physiological phenomenon for the functional balance of the coagulation–fibrinolytic system. In the pathogenesis of ACI, platelet activation is a key factor, and the increase in coagulation factors is the determinant of thrombosis.

Further analysis showed that there was a positive correlation between control group cases and patients group subjects on the expression of CD63, CD62P, and PAI-1. It is suggested that CD63, CD62P, PAI-1 is likely to participate in the pathogenesis of ACI in patients. Early diagnosis and evaluation of the disease have important value. In addition, the expression of CD63, CD62P, and PAI-1 was significantly higher in other subtypes of cerebral infarction patients than that of lacunar infarction cases. Previous studies have shown that lacunar infarction and other types of cerebral infarction may be different in etiology.[8] Therefore, the increase of CD63, CD62P, and PAI-1 expression may be more important in other types of cerebral infarction.

In summary, CD63, CD62P, and PAI-1 are highly expressed in PBMC and serum of patients with ACI, which may be closely related to the occurrence and development of patients with ACI. These indices may be used as indicators of clinical diagnosis and prognosis in patients with ACI. However, due to the small sample size of this study, it is necessary to carry out multi-center large-sample study in the future.

Acknowledgements

Zhanyun Ren contributed equally to this research and should be considered as co-first author.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Dong XL, Xu SJ, Zhang L et al. (2017) Serum resistin levels may contribute to an increased risk of acute cerebral infarction. *Molecular Neurobiology* 54(3): 1–8.
2. He X, Li DR, Cui C et al. (2017) Clinical significance of serum MCP-1 and VE-cadherin levels in patients with acute cerebral infarction. *European Review for Medical and Pharmacological Sciences* 21(4): 804–808.
3. Holven KB, Aukrust P, Pedersen TM et al. (2007) Enhanced platelet activation in hyperhomocysteinemic individuals. *Journal of Thrombosis and Haemostasis* 5(1): 193–195.
4. Dimopoulou I, Daganou M, Dafni U et al. (1998) Phrenic nerve dysfunction after cardiac operations: Electrophysiologic evaluation of risk factors. *Chest* 113(1): 8–14.
5. Chen J, Zhang C, Jiang H et al. (2005) Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. *Journal of Cerebral Blood Flow and Metabolism* 25(2): 281–290.
6. Choudhury A, Chung I, Blann AD et al. (2007) Platelet surface CD62P and CD63, mean platelet volume, and soluble/platelet P-selectin as indexes of platelet function in atrial fibrillation: A comparison of "healthy control subjects" and "disease control subjects" in sinus rhythm. *Journal of the American College of Cardiology* 49(19): 1957–1964.
7. Yoshida Y, Yamada M, Wakabayashi K et al. (1989) Endothelial basement membrane and seamless-type endothelium in the repair process of cerebral infarction in rats. *Virchows Archiv. A, Pathological Anatomy and Histopathology* 414(5): 385–392.
8. Arboix A, Font A, Garro C et al. (2007) Recurrent lacunar infarction following a previous lacunar stroke: A clinical study of 122 patients. *Journal of Neurology, Neurosurgery, and Psychiatry* 78(12): 1392–1394.