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Original Article

Ginkgo biloba Damo injection combined with troxerutin regulates the TLR4/NF-κB pathway and promotes the recovery of patients with acute cerebral infarction

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Abstract: Objective: Analysis of the effect of Ginkgo biloba Damo injection combined with troxerutin on patients with acute cerebral infarction (ACI) by regulating the TLR4/NF-κB signaling pathway. Methods: 150 patients with ACI were recruited for a prospective study. These patients were randomly divided into Ginkgo biloba Damo treatment group (n=75) and combined treatment group (Ginkgo biloba Damo + troxerutin, n=75) bilobabiloba. The patients in both groups were treated with conventional treatment. In addition, patients in the Ginkgo biloba Damo treatment group were treated with Ginkgo biloba Damo injection, and those in the combined treatment group were treated with Ginkgo biloba Damo injection combined with troxerutin. The National Institutes of Health Stroke Scale (NIHSS) and Barthel Index (BI) of the two groups were compared. The serum levels of GSH-Px, OX-LDL, CAT, TLR4, NF-κB p65, TNF-α, IL-1β and IL-6 were detected. The therapeutic effects of the two groups were compared. Results: After treatment, compared with the Ginkgo biloba Damo group, the combined treatment group had a lower NIHSS score and lower expression levels of OX-LDL, TLR4, NF-κB p65, TNF-α, IL-1β, and IL-6, but higher Barthel score and total effective rate as well as higher serum levels of GSH-Px and CAT (all $P<0.05$). Conclusion: Ginkgo biloba Damo injection combined with troxerutin can improve the neurologic function and activities of daily life in patients with ACI, which can also regulate the TLR4/NF-κB signaling pathway and downstream inflammatory factors to reduce brain tissue inflammation and oxidative stress damage.

Keywords: Acute cerebral infarction, Ginkgo biloba Damo injection, troxerutin, neurologic function

Introduction

Cerebral infarction, also known as stroke, is a common clinical brain disease [1]. Most of the patients with cerebral infarction have acute cerebral infarction (ACI). The onset of the disease is relatively sudden with the characteristics of high severity and high mortality [2]. The incidence of ACI is higher in the elderly. With the development of the aging population in China, the incidence of ACI remains high, and the number of new cases of ACI increases yearly. This has attracted the attention of scholars [3, 4]. If the ACI patients do not receive effective treatment, it will be complicated with a series of symptoms, seriously threatening the prognosis. Therefore, increasingly,experts and scholars have been exploring the clinical treatment of ACI.

Ginkgo biloba Damo injection has the effects of dilating cerebral blood vessels and inhibiting platelet aggregation. It is often used in the treatment of neurologic diseases. Studies have shown that Ginkgo biloba Damo injection can be used in the treatment of Alzheimer's disease, and can regulate the levels of TNF-α, IL-1β, and IL-6 in the downstream factors of the TLR4/NF-κB signaling pathway to reduce the inflammatory reaction of brain tissue and nerve damage [5]. Troxerutin has a certain protective effect on ischemic brain injury, and it can also be used in the treatment of cerebral infarction [6]. At present, there are few reports on the clinical study of Ginkgo biloba Damo injection combined with troxerutin in the treatment of ACI. Therefore, in this study, we used Ginkgo biloba Damo injection combined with troxerutin to treat patients with ACI to observe its effect.

Materials and methods

Materials

In this prospective study, 150 patients with ACI treated in Jinling Hospital from March 2019 to March 2020 were included and divided into ginkgo dipyridamole group (n=75) and combined treatment group (n=75) according to the random number table method. All the patients' family members had signed the informed consent form. This study was approved by the Ethics Committee of Jinling Hospital.

Inclusion criteria: all patients met the diagnostic criteria of ACI of Chinese Medical Association [7]. They were confirmed by imaging examination in Jinling Hospital and had been admitted to hospital for treatment within 24 hours of onset.

Exclusion criteria: 1) Patients had incomplete medical records; 2) Patients who were allergic, intolerances or contraindicated to the drugs in this study; 3) Patients with severe dysfunction of heart, kidney, lung and other organs; 4) Patients with cerebral hemorrhage; 5) Patients with malignant tumors of the head and neck.

Methods

Treatment method: Both groups of patients were treated by thrombolysis, and were given conventional treatments such as oxygen inhalation, lowering of intracranial pressure, controlling blood pressure, improving microcirculation, and maintaining water and electrolyte balance.

After thrombolytic therapy, patients in the Ginkgo biloba Damo group were treated with Ginkgo biloba Damo injection (Tonghua Guhong Pharmacy Co., Ltd., China. Batch number: H20181101): 30 mL of Ginkgo biloba Damo injection was added to 250 mL normal saline, and it was injected by intravenous drip once a day.

The combined treatment group was treated with troxerutin (Shandong Xinhua Pharmaceutical Co., Ltd., China. Batch number: H2019-0201) on the basis of the Ginkgo biloba Damo group. The 250 mL of troxerutin hydrolysate injection was diluted in 10 mL sodium chloride injection, and it was injected to the patients by intravenous drip once a day.

The patients in both groups were treated continuously for 14 days, and the effect was observed.

The scores of National Institutes of Health Stroke Scale (NIHSS) and Barthel score: The NIHSS was used to evaluate the neurological function of the patients before and after treatment [8]. High score indicates serious neurological impairment of patients. The scores of modified BI were used to evaluate the activities of daily living of the patients in the two groups, including defecation control, dressing, eating, bathing, walking the stairs, going to the toilet, and washing, with a full score of 100, and a high score indicates good ability of daily living [9].

Detection of serum GSH-Px, OX-LDL and CAT levels by immune transmission turbidimetry: 5 mL of fasting venous blood was collected from all patients in the acute stage (2 weeks after onset) and 2 weeks after treatment. The blood was stored at -80°C after centrifugation. Serum was collected to detect the levels of GSH-Px, OX-LDL, and CAT by standard curve method. The spectrophotometer was used to compare the color at 500 nm wavelength.

Enzyme linked immunosorbent assay (ELISA) was used to detect the expression of serum TLR4, NF-κB p65, TNF-α, IL-1β, and IL-6: About 5 mL of fasting venous blood was collected from all patients in the acute stage (2 weeks after onset) and 2 weeks after treatment. The blood was stored at -80°C after centrifugation. The serum was collect to detect the levels of TLR4, NF-κB p65, TNF-α, IL-1β and IL-6 according the kit instructions. The ELISA kits were purchased from Shanghai enzyme-linked Biotechnology Co., Ltd., China.

Evaluation criteria of therapeutic effect: Two weeks after the treatment, the therapeutic effect of the patients was evaluated according to the criteria of basic healing, markedly effective, effective and ineffictive. Basic healing: NIHSS score had dropped by more than 90%. Markedly effective: the NIHSS score was dropped by 45% to 90%. Effective: NIHSS score was decreased by 18% to 44%. Ineffective: the NIHSS score decreased by less than 18%. The overall response rate (%) = (basic healing cases + markedly effective cases + effective cases)/ total number of cases × 100.

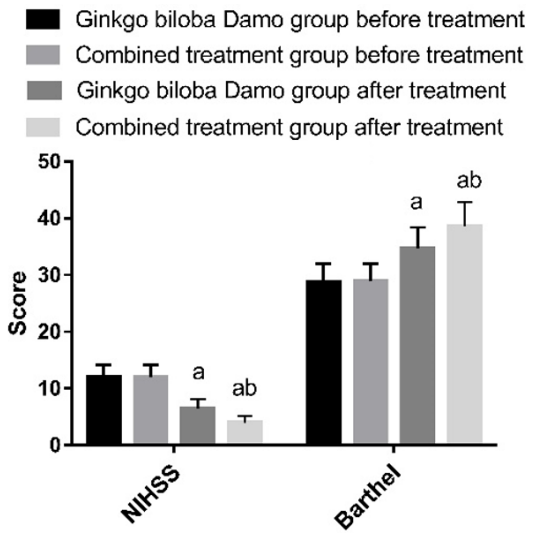

Figure 1. Comparison of NIHSS and BI scores between the two groups before and after treatment. NIHSS: National Institutes of Health Stroke Scale; BI: Barthel Index. Compared with before treatment, [a]P<0.05; compared with Ginkgo biloba Damo group, [b]P<0.05.

Statistical processing

Statistical analysis was conducted by SPSS 21.0 software. The enumeration data were expressed by %, and the comparison between groups was done by χ^2 test. The measurement data were expressed as mean ± ($\bar{x}$ ± sd); paired sample t-test was used for intra-group comparison, and independent sample t-test was used for inter-group comparison. P<0.05 was considered significant.

Results

Baseline data

The Ginkgo biloba Damo treatment group included 41 males and 34 females, aged from 62 to 73 years old, with an average of 67.5±4.4 years old. The combined treatment group included 45 males and 30 females, aged from 60 to 72 years, with an average of 65.9±4.9 years old. There was no statistical difference in the baseline data between the two groups (all P>0.05).

The combined treatment could improve the neurologic function and daily living ability

As shown in **Figure 1**, there was no significant difference in the scores of NIHSS and BI between the two groups before treatment (P>0.05). After treatment, the NIHSS score of the two groups decreased and the Barthel score increased (P<0.05). Compared with the Ginkgo biloba Damo group, the NIHSS score was lower and the Barthel score was higher in the combined treatment group (P<0.05).

The combined treatment could reduce the severity of brain oxidative stress injury in patients with ACI

As shown in **Table 1**, there was no significant difference in the levels of GSH-Px, OX-LDL, and CAT between the two groups before treatment (P>0.05). After treatment, compared with Ginkgo biloba Damo group, the combined treatment group had higher levels of serum GSH-Px and CAT, and lower level of OX-LDL (P<0.001).

The combined treatment group had significantly lower expression levels of TLR4 and NF-κB p65 after treatment

As shown in **Figure 2**, when comparing the expression levels of TLR4 and NF-κB p65 between the two groups before treatment, there were no statistical differences (P>0.05). After treatment, when compared with Ginkgo biloba Damo group, the combined treatment group had significantly lower expression levels of TLR4 and NF-κB p65 (P<0.05).

The combined treatment group had significantly lower levels of inflammatory factors after treatment

As shown in **Figure 3**, before treatment, when comparing the levels of TNF-α, IL-1β and IL-6 between the two groups, the difference was not significant (P>0.05). After treatment, the levels of serum TNF-α, IL-1β and IL-6 in the combined treatment group were significantly lower than those in the Ginkgo biloba Damo group (P<0.05).

The combined treatment group had significantly better therapeutic effects

As shown in **Table 2**, compared with the Ginkgo biloba Damo group, the combined treatment group had a higher overall response rate (P< 0.05).

Discussion

Cerebral infarction is cerebral infarction, also known as ischemic stroke, is a common ner-

Table 1. Comparison of GSH-Px, OX-LDL, and CAT levels before and after treatment in the two groups ($\bar{x} \pm sd$)

Group	Ginkgo biloba Damo group	Combined treatment group	t	P
GSH-Px (U/L)				
Before treatment	55.86±6.13	55.92±6.05	0.060	0.952
After treatment	62.99±6.78	68.57±7.13	4.912	<0.001
t	6.756	11.720		
P	<0.001	<0.001		
OX-LDL (μg/L)				
Before treatment	609.88±69.85	611.33±71.25	0.126	0.900
After treatment	551.27±60.23	472.35±52.33	8.566	<0.001
t	5.503	13.620		
P	<0.001	<0.001		
CAT (U/mL)				
Before treatment	50.24±5.34	50.19±5.27	0.058	0.954
After treatment	62.37±6.22	70.35±6.79	7.505	<0.001
t	12.810	20.310		
P	<0.001	<0.001		

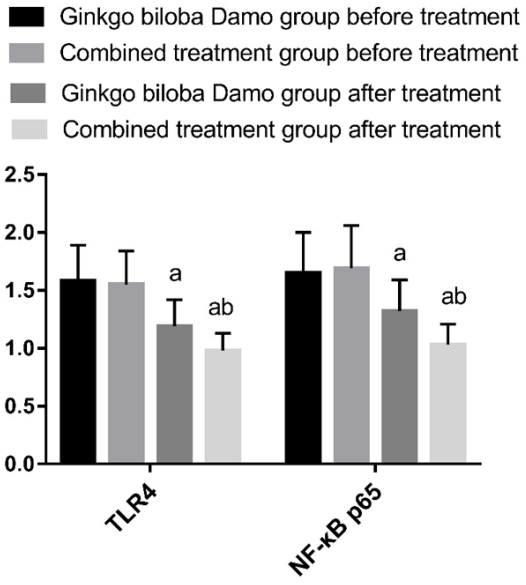

Figure 2. Comparison of TLR4 and NF-κB p65 expression. TLR4: toll like receptor 4; NF-κB p65: nuclear transcription factor kappa B p65. Compared with before treatment, [a]P<0.05; compared with Ginkgo biloba Damo group, [b]P<0.05.

vous system disease. It is a symptom of neurological deficit caused by insufficient blood supply to brain tissue, ischemia, and hypoxic necrosis [10, 11]. ACI has the characteristics of sudden onset and serious condition, the main manifestations of which are headache, dizziness, and hemiplegia. This seriously threatens the safety of patients, so it is of great significance to find a safe and effective treatment [12, 13]. The main components of Ginkgo ginkgo Damo injection include total flavone of ginkgo and dipyridamole, etc. Among these, the total flavonoids of ginkgo have the effect of dilating the blood vessels of brain tissue and improve the ischemic injury of brain tissue. Dipyridamole has the effect of inhibiting platelet aggregation [14]. Troxerutin can prevent thrombosis and has a certain protective effect on ischemic brain injury [15].

A large number of clinical studies have shown that the occurrence and development of ACI symptoms causes serious damage to the neurological function of patients, thus affecting activities of daily living [16-18]. The results showed that Ginkgo biloba Damo injection combined with troxerutin could significantly reduce the neurologic deficit and improve the ability of daily living in patients with ACI. The reason for this result may be that Ginkgo biloba Damo injection combined with troxerutin can improve the cerebral blood flow of patients with ACI, so as to alleviate the neurologic deficit and improve the daily living of patients.

The occurrence and development of ACI is closely related to the oxidative stress response. Reducing oxidative stress injury can reduce the severity of neurologic damage in patients with ACI, which is the key to treat ACI and improve the prognosis of patients with ACI [19, 20]. GSH-Px, OX-LDL and CAT are commonly used as clinical indicators to evaluate the antioxidant capacity of the body, and the changes in the three levels are closely related to the imbalance of the antioxidant defense system and the severity of oxidative stress injury [21]. Zhou et al. found that dyslipidemia and oxidative stress reaction are aggravated in patients with ACI complicated with Hp infection, which may be the cause of aggravated ACI disease in the patients [22]. The results of this study showed that the levels of GSH-Px and CAT increased and OX-LDL decreased in patients with ACI treated with Ginkgo biloba Damo injection

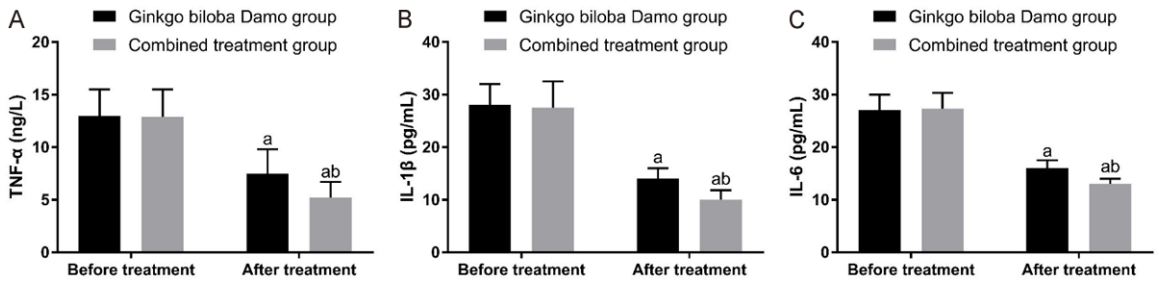

Figure 3. Comparisons of TNF-α, IL-1β, and IL-6 levels. A: TNF-α level; B: IL-1β level; C: IL-6 level. TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; IL-6: Interleukin-6. Compared with before treatment, [a]P<0.05; compared with Ginkgo biloba Damo group, [b]P<0.05.

Table 2. Comparison of therapeutic effects (n, %)

Groups	n	Basic healing	Markedly effective	Effective	Ineffective	Overall response rate
Ginkgo biloba Damo group	75	14 (18.67)	25 (33.33)	20 (26.67)	16 (21.33)	59 (78.67)
The combinedtreatment group	75	24 (32.00)	23 (30.67)	22 (29.33)	6 (8.00)	69 (92.00)
χ^2			7.356			5.327
P			0.061			0.021

combined with troxerutin, suggesting that the combination of the two can reduce the severity of brain oxidative stress injury in patients with ACI, and it plays an important role in relieving neurological damage in patients with ACI.

The TLR4/NF-κB signal pathway is involved in inflammatory reaction, apoptosis, and other pathologic processes. Some studies have shown that the TLR4/NF-κB signal pathway plays an important role in the occurrence and development of ACI [23, 24]. TLR4 is expressed in cerebral vascular cells. After ACI, the blood and oxygen supply of brain tissue are insufficient, the permeability of cerebral vascular endothelium is changed, and TLR4 is released to blood, which promotes an inflammatory reaction of brain tissue [24]. TNF-α, IL-1β and IL-6 are all downstream factors of TLR4/NF-κB signal pathway, and their levels change with the expression of TLR4/NF-κB signal pathway proteins, which participate in the inflammatory response of the body [25]. Guo et al. reported that Picroside 2 could down-regulate the expressions of TLR4, NF-κB and TNF-α to inhibit apoptosis and inflammation induced by cerebral ischemic reperfusion injury and improve the neurobehavioral function of rats [26]. The results of Ma et al. indicated that treadmill training promoted functional recovery and reduced the overexpression of TLR2, TLR4, NF-κB, and MyD88 in rat brain tissue after ischemia [27]. The results of this study showed that the expression of TLR4 and NF-κB p65 and the levels of TNF-α, IL-1β, and IL-6 decreased in patients with ACI treated with Ginkgo biloba Damo injection combined with troxerutin, indicating that the combination of the two can regulate the signaling pathway of TLR4/NF-κB and thus regulate the levels of downstream inflammatory factors TNF-α, IL-1β, and IL-6. It plays an important role in improving the inflammatory reaction of brain tissue in patients with ACI, and it is of great significance to the improvement of neurologic function in patients with ACI.

However, the number of cases selected in this study is limited, and the patients were not followed up for a certain period of time after treatment and discharge, and the prognosis of the patients could not be studied. The next step is to increase the sample size and follow-up time to benefit the study of the effect of this treatment on the prognosis of patients with ACI.

In conclusion, Ginkgo biloba Damo injection combined with troxerutin has a definite therapeutic effect on ACI, which can improve the neurologic function, activities of daily life, and blood coagulation in patients with ACI. Its function may be related to the regulation of TLR4/

NF-κB signaling pathway and downstream inflammatory factors, reducing the inflammatory reaction of brain tissue, and reducing oxidative stress injury, which provides a theoretical basis for the clinical treatment of ACI.

Disclosure of conflict of interest

None.

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