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*Original Article*

# Influence of Naodekang on serum cytokines in patients with acute ischemic stroke

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**Abstract:** Objective: This study aimed to explore the effect of Naodekang on acute Ischemic Stroke. Methods: 362 patients with acute ischemic stroke hospitalized in our neurosurgery department from Jan 2009 to Dec 2014 were randomly divided into treatment group and control group. The control group was given conventional medical treatment, and treatment group was given 9 g of Naodekang po. (or through NG-tube) tid. besides conventional treatment for 14 days. Patients in these groups regarding their neural dysfunction before and after treatment as well as the changes in the levels of MMP-2, MMP-9, IL-8 and hs-CRP were scored. Results: Treatment group showed significant improvement in neural dysfunction scores ($P < 0.01$) compared with control group. In addition, the levels of MMP-2, MMP-9, IL-8 and hs-CRP were decreased significantly after treatment ($P < 0.01$) compared with control group. Conclusion: Naodekang probably improved neural function of patients with acute ischemic stroke by altering their serum level of MMP-2, MMP-9, IL-8 and hs-CRP.

**Keywords:** Naodekang, acute ischemic stroke, MMP-2, MMP-9, IL-8, hs-CRP

## Introduction

Cerebrovascular disease is the commonest and frequently-occurring neural disease, characteristic of high morbidity, disability, and a mortality rate of about 10%, making it one of the three fatal threats to human health [1, 2]. 50%-70% of the survivors had sequelae to different extents, and 70% of cerebral strokes are ischemic. Some researches implied that acute ischemic stroke (AIS) has heightening effect on MMP-2, 9, IL-8 and hs-CRP levels in patient's serum, and relates positively to the degrees of insult [3]. Naodekang (Standard No: Lu Pharmaceutical ZBZ0054) was provided by Shandong Jiaozhou People's Hospital, which contains 11 kinds of herbal medicinesastragalus mongholicus, angelica, safflower, radix paeoniae rubrathe, Sichuan dome, salvianolic, lignum millettiae, etc., and it functions such that benefits qi for promoting blood circulation, removes obstruction in collaterals and blood stasis, suppresses hyperactive liver for calming endogenous wind and resolving convulsion, relieves five consumptions and seven damages, dredges channels for resuscitation, etc. This research aimed merely at its possible effect on the neural dysfunction in patients with AIS.

## Methods

### *Clinical materials*

362 AIS patients were selected as participants who were hospitalized within 24 hours after paroxysm between Jan 2009 and Dec 2014, and who met the diagnostic criteria by fourth National Conference on Cerebrovascular Diseases [4]. TCM diagnosis was on the basis of the Guiding Principles of Clinical Research on New TCM Drugs. Patients were excluded if they had cerebral tumor, cerebral trauma, autoimmune diseases, metabolic diseases, peripheral vascular embolism and hepatic or renal insufficiency. They were then divided into treatment group and control group, with 181 cases in either group. There were no significant differences between these two groups in terms of the condition, CT signs, clinical symptoms and complications, etc. (**Table 1**, $P > 0.05$).

### *Treatment*

The control group was given conventional treatment, oxygenation, respiratory support, dehydration to relieve intracranial pressure, antiplatelet aggregation, cerebral circulation im-

**Table 1.** Comparison between conditions of two groups

| Conditions | | Control Group (*n*=181) | Treatment Group (*n*=181) |
|---|---|---|---|
| Sex/Cases | Male:Female | 102:79 | 98:83 |
| Age/Years | | 58±6 | 59±6 |
| Radiographic Infarction/Cases | Basal Ganglion Region | 92 | 95 |
| | Temporal Lobe | 19 | 21 |
| | Occipital Lobe | 14 | 10 |
| | Multiple | 56 | 56 |
| GCS score/Cases | 3-8 points | 38 | 41 |
| | > 8 points | 143 | 140 |
| Preoperative Pupillary Changes/Cases | | 6 | 7 |
| Hemiplegia/Cases | Complete | 49 | 51 |
| | Incomplete | 132 | 130 |
| Hypertension/Cases | | 96 | 101 |
| Diabetes/Cases | | 22 | 17 |
| Rheumatic Heart Disease/Cases | | 9 | 11 |
| Atrial Fibrillation/Cases | | 21 | 19 |

P > 0.05, According to comparison with control group by t and $\chi^2$ test.

**Table 2.** NIHSS Score Comparison

| Group | Cases | Before Treatment | After Treatment |
|---|---|---|---|
| Control | 181 | 22.64±4.66 | 15.08±4.71 |
| Treatment | 181 | 21.73±4.58 | 8.70±3.83 |

provement, and cerebrocellular alimentation; for those who presented with operative indications, the pressure relief by craniotomy was given. The treatment group was given 9 g of Naodekang (Lu Pharmaceutical ZBZ0062, 90 g a bottle) po (or through NG-tube) tid for two weeks on the basis of the treatments of control group.

Neural function was evaluated according to National Institute of Health Stroke Scale (NIHSS) [5]; the levels of MMP-2, MMP-9, IL-8 and hs-CRP were tested through cubital phlebotomy from fasted patients in the early morning before and after treatment.

*Animals study*

21 New Zealand rabbits, half male and half female, weighing 20-30 kg, provided by Qingdao Drugs Control Center were also employed in this study. 21 rabbits were divided randomly into three groups: control, high dosage and low dosage. Each group was given one dose a day, 6.39 g/kg for low dosage and 31.95 g/kg for high dosage (tantamount to 10 or 50 times as much as human dosage), and control group was given distilled water at the same volume. Record the administration within a week, measure cerebral blood flow by Color Doppler before experiment and after a week of administration in order to observe the influence of Naodekang on blood flow.

*Statistical analysis*

The data were put into SPSS 17.0 for processing, where normally distributed measurement data were marked as ($\bar{x}\pm s$) and tested by t test, while count data were tested by $\chi^2$ test, with P < 0.05 standing for significant difference.

## Results

*Comparison between two groups with reference to the neural function before and after treatment (NIHSS score)*

There was no significant difference in neural dysfunction between the two groups before treatment (P > 0.05), and there was significant decrease in the dysfunction after treatment in both groups (P < 0.01). The difference was significant between the two groups (P < 0.01) (**Table 2**).

There was significant difference between patients before and after treatment within each

**Table 3.** Serum level comparison of proteins

| Group | Cases | | hs-CRP (mg/L) | MMP-2 (μg/L) | MMP-9 (μg/L) | IL-8 (ng/L) |
|---|---|---|---|---|---|---|
| Control | 181 | Before | 21.75±7.91 | 401.54±78.26 | 431.26±96.74 | 38.64±11.28 |
| | | After | 8.97±4.22 | 315.36±81.38 | 241.1±67.46 | 26.27±8.68 |
| Treatment | 181 | Before | 21.88±8.02 | 398.61±85.37 | 430.87±98.57 | 38.79±10.62 |
| | | After | 7.65±3.83 | 256.59±81.62 | 201.2±64.96 | 20.87±9.35 |

P < 0.01 according to comparison between patients before and after treatment within each group; P < 0.01 according to comparison between two groups.

**Table 4.** The cerebral blood flow by Color Doppler before experiment and after a week of administration

| Group | Drug dosage (g/kg) | Animal Counts (Heads) | cerebral blood flow (Color Doppler, cm/s) | |
|---|---|---|---|---|
| | | | Before experiment | After a week of administration |
| Control | -- | 7 | 161.3±22.7 | 163.4±23.9 |
| Low dosage | 6.39 | 7 | 166.3±25.4 | 189.6±21.0 |
| High Dosage | 31.95 | 7 | 158.8±24.2 | 198.3±20.5 |

group (P < 0.01) and between control and treatment groups (P < 0.01).

*Comparison between two groups regarding serum levels of MMP-2, MMP-9, IL-8 and hs-CRP before and after treatment*

There was no significant differences in serum levels of MMP-2, MMP-9, IL-8 and hs-CRP between the two groups (P > 0.05) before treatment, and there was significant decrease in these levels after treatment in both groups (P < 0.01). The difference was significant between the two groups (P < 0.01) (**Table 3**).

*The measurement of cerebral blood flow by Color Doppler*

As shown in **Table 4**, Control group showed no changes, high and low dosage (6.39, 19.17 g/(kg.d)) groups both showed increases in cerebral blood flow.

## Discussion

Acute Ischemic Stroke (AIS) is neural symptoms or signs that stem from cerebral arterial sclerosis or stenosis resulting in local ischemia, hypoxia and necrosis [6]. The occurrence and progression of AIS were associated closely with the instability of atherosclerosis, during which inflammatory reaction is major in that the highly-concentrated inflammatory cytokine was possibly involved in neural impairment [7].

CRP is a kind of acute phase reaction protein (APRP) synthesized by hepatic cells, a sensitive indicator of nonspecific inflammatory reaction [8]. After haemorrhagic stroke, cytokines such as TNF-α and IL-β are released by activated vascular endothelial cells, astrocytes, and surrounding inflammatory cells resulting from severe ischemic and reperfusion injury in necrotic areas, which stimulates hepatic synthesis by inflammatory reaction [9, 10]. It has been proved by previous study that there is discernible increase in CRP level in patients with AIS, indicating the close connection between CRP and occurrence or progression of AIS, and making it a considerable biological index of the evaluation of AIS progression and prognosis [11]. MMP-9 is a set of proteinase secreted by mononuclear macrophage that decomposes extracellular matrix and relates closely to blood-brain barrier damage, released on occasion of cytokines by neural inflammation [12]. MMP-2 can decompose Type I and Type II collagen, break blood-brain barrier, and accelerate the crumbling of vulnerable plaques [13]. MMP-9 is a significant proteinase in extracellular matrix metabolism that decomposes extracellular matrix and ruins basement membrane, therefore causing thinning and rupture of fibrous cap, then accelerating embolism, which enhances permeability of blood-brain barrier and induces secondary injury after cerebral ischemia [14]. MMP-9 can ruin blood-brain barrier by increasing its permeability, aggravating cerebral edema and possibly attenuating the protection of fibrous cap.

The study revealed that MMP-2, 9 levels significantly increased after cerebral infarction, and was connected closely with the area of infarc-

tion and the level of consciousness disturbance. IL-8 is a potential neutrophil chemokine that influences neutrophils by chemotaxis and activationin early neural inflammatory reaction. It has been implied in certain study that IL-8 inhibitor could distinguishably reduce infarction area and edema degree.

Cerebral infarction is classified as "stroke" in TCM, considered to be caused by primordial qi deficiency and pathogenic qi stasis, or hepatic blood stasis and qi deficiency from channel blockade [15, 16]. The brain is the main focus involving spleen, stomach, liver, kidney and heart. The evident blood stasis arises essentially from qi deficiency. In TCM it is held that qi is commander of blood, and that blood goes as qi goes; blood stops as qi stops [17]. Stroke can be resolved by invigorating qi, removing stasis and dredging channels. Naodekang is based on altered buyang huanwu decoction, in which astragalus mongholicus serves as sovereign drug that invigorates spleen and stomach to benefit qi and circulate blood, then to remove stasis and dredge channels; angelica serves as minister drug that circulates and nourishes blood to remove stasis without harming blood; radix paeoniae rubrathe, sichuan dome, taoren and safflower serve as assistant drug to assist in circulating blood and removing stasis; dilong and chuanniuxi serve as envoy drug to dredge channels, and tianma calms endogenous wind and relieves spasm in collaboration with the other two envoy drugs to benefit qi, remove stasis and dredge channels [18-20]. In modern pharmacology, it is accepted that astragalus mongholicus can obviously dilate blood vessels, improve microcirculation and capillary resistance by prevention from the increase in brittleness and permeability, as well as inhibit ADP-induced platelet aggregation; angelica can inhibit phosphorylation of phosphatidylinositol in platelet membrane to protect against platelet aggregation; ligustrazine can pass rapidly through blood-brain barrier to dilate blood vessels, improve microcirculation and inhibit platelet aggregation in cerebral part; the effect of sichuan dome is enhanced by the synergy with Salvianolic, safflower and radix paeoniae rubrathe in regard to protection against embolismby dilating cerebral blood vessels largely and increase cerebral blood flow persistently, and it also inhibits platelet aggregation; dilong is rich in plasmin and plasminogen activator that resolve embolism by way of relieving inflammatory reaction connected with CRP, and that improve hemorheology by decreasing blood consistency, and to supply the tissue repair of ischemic area by reducing cerebral necrosis and accelerating blood circulation. It has been proved by modern biochemical methods that Naodekang can decrease significantly the levels of MMP-2, MMP-9 and IL-8, benefiting the recovery of neural function.

## Conclusion

This study revealed that treatment group had significantly higher NIHSS scores after 14 days than control group ($P < 0.05$), which means Naodekang could improve neural function of AIS patients. The levels of hs-CRP, MMP-9, IL-8 decreased significantly after treatment, implicating that Naodekang could relieve neural injury from inflammatory proteinase and inflammatory cytokine and therefore accelerate the recovery, which is a possible mechanism of AIS treatment.

## Disclosure of conflict of interest

None.

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