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Original Research Article

Efficacy of combined atorvastatin calcium, *Salviae miltiorrhizae* and ligustrazine hydrochloride injection in cerebral infarction patients

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Abstract

Purpose: *To study the effect of a combination of atorvastatin calcium with Salviae miltiorrhizae and ligustrazine hydrochloride injection on serum levels of ferritin (SF), C-reactive protein (CRP) and hypoxia-inducible factor 1α(HIF-1α) in cerebral infarction patients.*
Methods: *A total of 60 cerebral infarction patients (confirmed by CT or MRI scan) were randomly assigned to control group and observation group (30 patients/group). Both groups received routine treatments. All patients took atorvastatin calcium, but those in the observation group were treated with Salviae miltiorrhizae and ligustrazine hydrochloride injection, in addition to atorvastatin hydrochloride for 14 days, with 7 days as treatment course. The levels of SF, CRP and HIF-1α were determined before and after treatment, to assess clinical efficacy and safety.*
Results: *In both groups, SF, CRP and HIF-1α levels were lower after treatment than before treatment ($p < 0.05$). NIHSS score and platelet activation indices were also significantly reduced, relative to control ($p < 0.05$).*
Conclusion: *The combination of atorvastatin calcium with Salviae miltiorrhizae and ligustrazine hydrochloride injection can control vascular inflammatory reactions by decreasing the levels of SF, CRP and HIF-1α. Thus, it may be beneficial in the clinical management of cerebral infarction.*

Keyword: *Cerebral infarction, Atorvastatin calcium, Salviae miltiorrhizae, Ligustrazine hydrochloride injection*

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INTRODUCTION

Stroke is one of the top three fatal diseases worldwide, and 87 % of stroke incidents are due to cerebral infarction [1]. Cerebral infarction is associated with high mortality and morbidity [2]. The predisposing factors for cerebral infarction are atherosclerosis, hypertension, diabetes, heart disease, smoking and alcohol consumption [3]. Although cerebral infarction usually occurs within the age range of 40 - 70 years old, its trend in recent years appear to involve younger individuals.

An epidemiological investigation has reported that the incidence of ischemic stroke in people

aged 15 to 45 years is approximately 2 - 10 persons in 100,000 people, and that the incidence of stroke in people under 40 years old accounts for 10 – 20 % of all strokes [4]. Cerebral infarction has acute onset, which makes the affected patients liable to sudden death. The aim of the present study was to investigate the efficacy of combination of atorvastatin calcium with *Salviae miltiorrhizae* and ligustrazine hydrochloride injection on vascular inflammatory reaction in cerebral infarction patients.

EXPERIMENTAL

General characteristics of subjects

A total of 80 cerebral infarction patients, confirmed by CT or MRI scan in Neurology Department of Affiliated Hospital of Shaoxing University, from November 2015 to October 2017, were randomly assigned to control group and observation group (30 per group). There were 21 males and 19 females in the observation group, aged 40 to 75 years (mean age = 52.6 ± 6.8 years). With respect to infarct sites, there were 13 cases in basal ganglia, 8 cases in frontal lobe, 4 cases in parietal lobe, and 5 cases in temporal lobe. In the control group, there were 25 males and 10 females aged 40 - 77 years (mean age = 53.4 ± 7.1 years), with 15 infarcts in basal ganglia, 7 in frontal lobe, and 4 each in parietal lobe and temporal lobe. There were no statistically significant differences in general data such as sex, age and infarct sites between two groups.

This research was approved by the Ethical Committee of Department of Hospital Management, Qinhuangdao Maternity and Child Health Hospital, Hebei, 066000, China (approval no. 3510 [5]), and was carried out according to the guidelines of Declaration of Helsinki promulgated in 1964 as amended in 1996.

Inclusion criteria: Patients who met the diagnostic criteria of cerebral infarction drafted by the Fourth National Conference on Cerebrovascular Diseases [6], and whose cases were confirmed by images, were included in the study. Other included patients were those whose attack occurred ≤ 72 h prior to hospital admission, and who were conscious; patients with platelet counts > 80 × 10^9/L; and patients who got ethical approval, signed informed consent, and voluntarily participated in the study.

Exclusion criteria: Patients with hemorrhagic cerebral infarction or transient cerebral ischemia were excluded, as well as patients with mental, cognitive, and articulation disorders or abnormalities; and patients with history of neurological impairment, stroke, or severe anemia. In addition, patients who were on antiplatelet, anticoagulant or thrombolytic drugs, and patients who reacted adversely to atorvastatin calcium, *Salviae miltiorrhizae* and ligustrazine hydrochloride injection were not included. Other excluded patients were those with vital organ dysfunction, and patients who were unable to complete the study due to poor compliance.

Treatment

All patients received comprehensive and routine therapies in accordance with their individual conditions. These therapies included reduction of intracranial pressure, protection of the brain, regulation of blood pressure and blood sugar, and correction of water-electrolyte disorders. The control group was given atorvastatin calcium (Beijing Jialin Pharmaceutical Corporation, GYZZ: H20093819), 40 mg at a time, by mouth, once per day. In addition to atorvastatin calcium, the observation group was given *Salviae miltiorrhizae* and ligustrazine hydrochloride injection (Guizhou Bait Pharmaceutical Co., Ltd., GYZZ: H52020959) i.e. 10 mL *Salviae miltiorrhizae* and ligustrazine hydrochloride injection diluted with 250 mL of 5 % glucose at a time, intravenous drip, once per day . A treatment course was seven days, and both groups were treated continuously for two treatment courses.

Observation indices

Serum ferritin (SF), C-reactive protein (CRP) and hypoxia-inducible factor 1α (HIF-1α)

Cubital venous blood (5 mL) was collected in the morning from patients in both groups before and after treatment. The blood samples were centrifuged at 3000 rpm for 15 min, and the serum samples were refrigerated at -80°C prior to use. Serum ferritin (SF) was determined using immuno-turbidimetric method in SA808 Automatic Biochemical Analyzer (Shanghai Yongchuang Medical Instrument Co., Ltd.). The assays for CRP and HIF-1α were carried out using ELISA double-antibody sandwich method, with kits obtained from Shanghai Bangyi Biotechnology Co., Ltd.

NIHSS score and platelet activation indexes

Neurological function was assessed using American National Institute of Health Stroke Scale (NIHSS) with 15 items. In this scale, the higher the score, the more severe the neurologic

impairment. All NIHSS assessments were conducted by the same neurological physician. Platelet agglutination was determined by transmission turbidimetric assay, while platelet granule membrane protein 140 (GMP-140), thromboxane B2 (TXB2), and platelet activating factor (PAF) were assayed by enzyme-linked methods with kits from Shanghai Univ-Biotechnology Co., Ltd.

Criteria for efficacy assessment

The efficacy of treatments in both groups was evaluated according to the efficacy assessment criteria of neurological impairment in stroke patients drafted by the Fourth National Conference on Cerebrovascular Diseases i.e. basically cured = decrease in NIHSS score ≥ 90 %, with no disability; excellent = decrease in NISS score > 45 % but < 90 %, with disability grades of I –III; effective = decrease in NIHSS score >17 % but ≤ 45 %; and ineffective = decrease in NIHSS score ≤ 17 % or an increase in NISS score. The therapeutic effect was calculated from the NISS classifications as in Eq 1 where TE is therapeutic effect, B is number of basically cured cases, E1 is number of excellent cases, E2 is number of effective cases, and T is (total number of cases.

$$TE (\%) = \{(B + E1 + E2)/T\}100 \quad \ldots\ldots\ldots\ldots (1)$$

All adverse events that occurred during the study were observed, recorded and treated effectively and symptomatically.

Statistical analysis

The data that conformed with normal distribution are presented as mean ± standard deviation (SD), while enumeration data are expressed as ratio. Inter-group comparison was done with *t*-test, while ranked data were analyzed using rank sum test. Ratios were compared using χ^2 test. All statistical analyses were done with SPSS software version 17.0. Statistical significance was assumed at $p < 0.05$.

RESULTS

Levels of SF, CRP and HIF-1α

The levels of SF, CRP and HIF-1α in the two groups were decreased after treatment ($p < 0.05$; Table 1). However, SF, CRP and HIF-1α levels in the observation group were lower than those in the control group ($p < 0.05$).

NIHSS score and platelet activation indices

There were significant post-treatment reductions in NIHSS score, platelet agglutination, GMP-140, TXB2 and PAF in both groups ($p < 0.05$; Table 2). However, the observation group had significantly lower levels of NIHSS score, platelet agglutination, GMP-140, TXB2 and PAF than those the control group ($p < 0.05$).

Treatment efficacy

Table 3 shows that at the end of the two treatment courses, the therapeutic effect in the observation group (86.67 %) was significantly higher than that in the control group (73.33 %) ($Z = -2.761$, $p = 0.006 < 0.01$).

Incidents of adverse effects

Two patients in the control group came down with nausea and vomiting during the treatment. However, the symptoms subsided gradually after timely and symptomatic therapies. There were no other adverse events.

DISCUSSION

At present, the key strategies for treating cerebral infarction in the clinic involve re-canalization of vessels in the infraction zone, protection of ischemic penumbra, reduction of infarct size and reduction of neuronal impairment, all aimed at quick recovery of cerebral neurological function [7].

Table 1: SF, CRP and HIF-1α levels

Group	Cases	Time	SF (ng/mL)	CRP (mg/dL)	HIF-1a (ng/mL)
Control group	30	Before treatment	269.88±36.07	6.18±0.81	1721.16±239.87
		After treatment	211.16±28.75*	3.76±0.52*	967.89±122.29*
Observation group	30	Before treatment	272.36±34.65	6.26±0.77	1754.28±246.86
		After treatment	182.81±22.69*#	2.19±0.28*#	564.39±76.58*#

Values are expressed as mean ± SD

Table 2: NIHSS score and changes in platelet indices

Group	Time	NIHSS (score)	Platelet agglutination (%)	GMP-140 (g/L)	TXB2 (ng/L)	PAF (10^{-9}mol/L)
Control group	Before treatment	23.79±3.19	80.46±9.58	45.52±5.97	91.18±10.94	12.42±1.46
	After treatment	16.66±2.09*	71.12±8.44*	37.45±4.28*	77.15±10.41	10.85±1.28*
Observation group	Before treatment	24.88±3.21	81.31±9.30	46.27±5.71	90.34±11.20	12.58±1.40
	After treatment	10.73±1.46$^{*\#}$	64.25±7.10$^{*\#}$	30.66±3.69$^{*\#}$	68.55±9.48$^{*\#}$	8.22±1.08$^{*\#}$

Values are expressed as mean ± SD

Table 3: Treatment efficacy for the two groups [n (%)]

Group	Cases	Almost recovery	Effective	Progress	Ineffective	Positive rate
Observation group	30	17(56.67)	4(13.33)	5(16.67)	4(13.33)	26(86.67)
Control group	30	6(20.00)	5(16.67)	11(36.67)	8(26.67)	22(73.33)
Z			-2.761			
P			0.006			

Atorvastatin calcium, a third generation drug commonly used in clinics, has better lipid-lowering effect than simvastatin, fluvastatin or any other drug, and it can hinder inflammation, reverse plaque formation, and ameliorate infarction-induced brain injury [8].

Salviae miltiorrhizae and ligustrazine hydrochloride injection are components of a compound preparation containing tetramethylpyrazine hydrochloride and tanshinol [9]. The preparation has been widely used for treating acute cerebral infarction. It brings about changes in hemodynamics, thereby improving microcirculation in the cerebral infarction zone. In addition, it protects the brain by inhibiting the activity of monoamine oxidase. It has been suggested that platelet activation indexes are closely associated with cerebral infarction, and so can be used as important indexes for monitoring the disease [10]. Currently, PAF is considered the strongest platelet agglutination agent, while GMP-140 is the most characteristic platelet activation marker. Being a metabolite of TXA2, the effects of TXB2 are similar to those of TXA2.

The findings of this study indicate that clinical efficacy was better in the observation group than in the control group, but platelet activation indexes and NIHSS score were much less in the observation group than in the control group. This finding suggests that the combination of atorvastatin calcium with *Salviae miltiorrhizae* and ligustrazine hydrochloride injection can effectively limit platelet agglutination in cerebral infarction patients and mitigate neuronal injury, thereby protecting brain tissues in the ischemic zone.

The low incidents of adverse effects indicates that the combination of atorvastatin calcium with *Salviae miltiorrhizae* and ligustrazine hydrochloride injection has high level of safety and very low level of adverse reactions. The levels of SF, CRP and HIF-1α were much lower in the observation group than in the control group, showing that the combination therapy can effectively decrease the concentrations of these parameters. Ferritin is one of the important indicators used for assessing iron storage in the body. It has been reported that elevated ferritin level is a major risk factor for progression of ischemic stroke [11]. Thus, ferritin is useful for predicting the progression of cerebral infarction. Ferritin produces numerous hydroxy radicals in reperfusion injury in the cerebral ischemic area, and it initiates lipid peroxidation-induced damage to cellular membranes [12]. The hydroxyl radicals also bring about oxidative damage that further destroys proteins and nucleic acids, eventually leading to brain edema and brain cell necrosis.

It has been reported that CRP, a sensitive and non-specific inflammatory response marker, is an independent risk factor for poor prognosis in patients with ischemic stroke [13]. Changes in CRP level are positively related to neurological symptoms in some experimental animals. Thus, these changes have been considered as early signs of cerebral tissue injury. The only transcription factor that can play an active role in specific hypoxia is HIF-1α [14]. It enhances apoptosis in ischemic and hypoxia brain tissues in acute cerebral infarction. Therefore, HIF-1α level can indicate the extent of ischemic penumbra in cerebral infarction and the severity of secondary ischemic injury. The levels of SF,

CRP and HIF-1α provide some theoretical basis for the ischemic conditions in intracerebral hemorrhage and hematoma.

The present study has established that the combination of atorvastatin calcium with *Salviae miltiorrhizae* and ligustrazine hydrochloride injection can effectively inhibit platelet agglutination and improve cerebral neurological function. Moreover, the combination treatment has a good clinical efficacy and high safety, and it brings about significant decreases in SF, CRP and HIF-1α levels.

CONCLUSION

The combination of atorvastatin calcium with *Salviae miltiorrhizae* and ligustrazine hydrochloride injection may control vascular inflammatory reactions by decreasing the levels of SF, CRP and HIF-1α. Thus, it may beneficial in the management of cerebral infarction.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Linlin Fu conceived and designed the study, Linlin Fu, Baojun Zhang, Xiaomin Qiu collected and analysed the data, Linlin Fu wrote the manuscript.

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