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Clinical study of adjuvant therapy on ischemic stroke with salviae miltiorrhizae and ligustrazine

Jing-Qin Tian[1*], Wei Zhang[2]

[1]*Seventh Department of Encephalopathy, Weifang Hospital of Traditional Chinese Medicine, Weifang 261041, Shandong, China*

[2]*Second Department of Encephalopathy, Weifang Hospital of Traditional Chinese Medicine, Weifang 261041, Shandong, China*

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ABSTRACT

Objective: To study the effect of adjuvant therapy with salviae miltiorrhizae and ligustrazine injection on neural function injury, degree of oxidative stress reaction, chemotactic factor and adhesion molecule in patients with ischemic stroke.
Methods: Patients with ischemic stroke admitted in our hospital from August 2013 to October 2015 were retrospectively analyzed and divided into conventional treatment (CT) group receiving conventional treatment and traditional Chinese medicine auxiliary (TCMA) group receiving adjuvant therapy with salviae miltiorrhizae and ligustrazine injection. After the treatment of the first month and the third month, serum was collected to detect the contents of nerve injury molecules, oxidative stress parameters, chemotactic factor and adhesion molecule.
Results: After the 4-week treatment, the contents of serum neuron specific enolase, S100 calcium-binding protein B, heart-type fatty acid binding protein, malondialdehyde, advanced oxidation protein products, 8-hydroxy-2'-deoxyguanosine, monocyte chemoattractant protein-1, CD40, CD40 ligand, vascular endothelial cadherin, soluble intercellular adhesion molecule 1 and soluble vascular adhesion molecule 1 in patients of TCMA group were all significantly lower than those of CT group, and the contents of superoxidase dismutase, glutathione peroxidase and catalase were significantly higher than those of CT group. After the 8-week treatment, the contents of serum neuron specific enolase, S100 calcium-binding protein B, heart-type fatty acid binding protein, malondialdehyde, advanced oxidation protein products, 8-hydroxy-2'-deoxyguanosine, monocyte chemoattractant protein-1, CD40, CD40 ligand, vascular endothelial cadherin, soluble intercellular adhesion molecule 1 and soluble vascular adhesion molecule 1 in patients of TCMA group were all significantly lower than those of CT group and the contents of superoxidase dismutase, glutathione peroxidase and catalase were significantly higher than those of CT group.
Conclusions: Adjuvant therapy with salviae miltiorrhizae and ligustrazine injection can alleviate the neural function injury, inhibit oxidative stress reaction and the generation of chemotactic factor and adhesion molecule in patients with ischemic stroke, which is an effective medicine for treating ischemic stroke.

1. Introduction

Ischemic stroke is a common cardiovascular and cerebrovascular disease in clinic, which mostly occurs in mid-aged population, causing ischemic hypoxia injury in neural function and affecting people's daily life. Modern medical research believes that atherosclerosis is the pathological basis for causing acute ischemic stroke[1-3]. In the pathological process of intracranial atherosclerosis, the gradual formation of plaque will lead to the vessel stenosis, decreasing blood flow volume and then the occurrence of eddy or turbulence. Plaque will rupture and its subintimal collagen exposes with the impact of eddy or turbulence, which increases the local adhesion of blood platelet, activates the blood platelet, forms local thrombosis, blocks blood vessels and then causes clinical characteristics of cerebral infarction[4,5]. In the treatment of ischemic stroke with western medicine, a commonly used method is to inhibit the activation of blood platelet by using aspirin and clopidogrel simultaneously with the adjuvant therapy of lipid-lowering and neurotrophic drugs[6-8]. However, due to the obvious increasing activation degree of blood platelet in patients with ischemic stroke, although the conventional antiplatelet therapy achieves curative effect to some extent, the risk of occurrence of re-

*Corresponding author: Jing-Qin Tian, Seventh Department of Encephalopathy, Weifang Hospital of Traditional Chinese Medicine, Weifang 261041, Shandong, China.
Tel: +86 15094997220
E-mail: celerytian2008@163.com

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infarction will be much greater in the long-term treatment process.

In recent years, traditional Chinese medicines are widely used in the treatment of acute cerebral infarction. Brain stoke is included in the category of stroke of traditional Chinese medicine theory. The prescription of promoting blood circulation to remove blood stasis can play a therapeutic role for the stroke of the pathogenesis of the disease. The compound preparation of salviae miltiorrhizae and ligustrazine is refined by traditional chinese medicinal materials, salvia miltiorrhiza and ligusticum wallichii whose effective constituents are salvianolate and ligustrazine respectively, which have an effect on promoting blood circulation to remove blood stasis strongly. Modern pharmacology study believes that salviae miltiorrhizae and ligustrazine have a function of anti-platelet aggregation and improving minicirculation. Also, they can eliminate the free radicals massively produced under the hypoxic condition, which offers a treatment value in most pathological links of ischemic stroke. In the following study, the effect of adjuvant therapy with salviae miltiorrhizae and ligustrazine injection on neural function injury, degree of oxidative stress reaction, chemotactic factor and adhesion molecule in patients with ischemic stroke was analyzed .

2. Materials and methods

2.1. Study subjects

Patients with ischemic stroke admitted in our hospital from August 2013 to October 2015 were considered as the study subjects. The inclusion criteria were as follows: (1) in accordance with the diagnostic criteria of cerebral infarction in Internal Medicine (eighth edition)[8] and that of stroke in TCM-SSASD[9], (2) treatment in hospital within 72 h after onset, (3) cerebral infarction lesions confirmed with head magnetic resonance imaging, and (4) acquiring informed consent. The exclusion criteria were as follows: (1) patients with transient ischemic attack; (2) patients merged with disturbance of consciousness, myocardial infarction, thrombotic diseases and coagulation disorders, (3) incomplete medical data, and (4) insufficient heart, liver and kidney functions of patients. There were totally 69 cases of patients. By retrospectively analyzing their medical data, these patients were divided into traditional Chinese medicine auxiliary (TCMA) group and conventional treatment (CT) group based on the treatment protocols.

2.2. Treatment methods

Patients of both groups were given symptomatic and supportive treatments such as maintaining the balance of water and electrolyte, dehydration, improvement of brain edema and anti-infection. Simultaneously, they were orally given 100 mg of aspirin enteric-coated tablets once a day, 75 mg of clopidogrel hydrogen sulfate tablets once a day and 20 mg of atorvaststin once a day. As for the patients of TCMA group, they were given an extra treatment of salviae miltiorrhizae and ligustrazine injection based on the CT mentioned above. The methods were as follows: 10 mL of salviae miltiorrhizae and ligustrazine injection added with 250 mL of normal saline was given by intravenous drip once a day for 8 weeks of continuous treatment.

2.3. Detection methods of serum index

A volume of 5 mL of peripheral blood specimens from both groups was collected in the 4th week and the 8th week of the treatment and centrifuged for serum. ELISA was used to determine the contents of S100 calcium-binding protein B (S100B), neuron specific enolase (NSE) and heart-type fatty acid binding protein (H-FABP), and monocyte chemoattractant protein-1 (MCP-1), vascular endothelial cadherin (VE-cadherin), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular adhesion molecule 1 (sVCAM-1). Radioimmune precipitation test kit was used to measure the contents of superoxidase dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA), adanced oxidation protein products (AOPP) and 8-hydroxy-2'-deoxyguanosine (8-OHdG).

2.4. Statistical methods

Software SPSS version 21.0 was used to input and analyze the data and *t*-test was used to analyze the measurement data between the two groups. Difference was considered statistically significant when $P < 0.05$.

3. Results

3.1. General data of both groups

There were 24 males and 14 females in TCMA group ($n = 38$) with the age of (61.30 ± 7.90) years and body mass index (BMI) of (23.77 ± 3.51) kg/m^2. It consisted of 21 cases of combining hypertension, 12 cases of diabetes and 22 cases with smoking history. In CT group, there were 20 males and 11 females ($n = 31$) with the age of (60.70 ± 8.10) years and BMI of (23.49 ± 3.24) kg/m^2. It included 19 cases of combining hypertension, 10 cases of diabetes and 19 cases with smoking history. By statistically analyzing, there was no significant difference of gender, age, BMI and case number for combining hypertension, diabetes and smoking history between two groups (Table 1).

3.2. Contents of markers of neural injury

In the 4th week of treatment, the contents of serum NSE [(7.24 ± 0.88) *vs.* (12.12 ± 1.76) ng/mL] and S100B [(0.88 ± 0.10) *vs.* (1.41 ± 0.19) ng/mL], H-FABP [(0.34 ± 0.06) *vs.* (0.51 ± 0.07) ng/mL] from TCMA group were significantly lower than those of CT group. In the 8th week of treatment, the contents of serum NSE [(5.51 ± 0.72) *vs.* (9.24 ± 1.09) ng/mL] and S100B [(0.62 ± 0.07) *vs.* (1.17 ± 0.16) ng/mL] and H-FABP [(0.31 ± 0.06) *vs.* (0.46 ± 0.08) ng/mL] from TCMA group were significantly lower than those of CT group. Difference of serum contents of NSE, S100B and H-FABP at 4th and 8th week of treatment from both groups was considered statistically significant ($P < 0.05$) (Table 2).

3.3. Parameters of oxidative stress

In the 4th week of treatment, the contents of serum SOD [(138.54

Table 1
Clinical data of subjects of two groups.

Group	Gender (male/female)	Age (year)	BMI (kg/m^2)	Hypertension	Diabetes	Smoking
TCMA group (n = 38)	24/14	61.30 ± 7.90	23.77 ± 3.51	21 (55.26%)	12 (31.58%)	22 (57.89%)
CT group (n = 31)	20/11	60.70 ± 8.10	23.49 ± 3.24	19 (61.29%)	10 (32.26%)	19 (61.29%)

Table 2
Comparison of severity of neural injury between two groups (ng/mL).

Group	The 4th week of treatment			The 8th week of treatment		
	NSE	S100B	H-FABP	NSE	S100B	H-FABP
TCM group (n = 38)	7.24 ± 0.88	0.88 ± 0.10	0.34 ± 0.06	5.51 ± 0.72	0.62 ± 0.07	0.31 ± 0.06
CT group (n = 31)	12.12 ± 1.76	1.41 ± 0.19	0.51 ± 0.07	9.24 ± 1.09	1.17 ± 0.17	0.46 ± 0.08
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

± 17.61) *vs.* (94.52 ± 10.15) IU/mL], GSH-Px [(168.65 ± 15.65) *vs.* (103.45 ± 11.52) IU/mL] and CAT [(75.68 ± 9.34) *vs.* (48.59 ± 6.41) IU/mL] from TCMA group were significantly higher than those of CT group and the contents of serum MDA [(4.85 ± 0.65) *vs.* (8.98 ± 1.03) μmol/L], AOPP [(60.34 ± 7.68) *vs.* (96.87 ± 11.36) μmol/L] and 8-OHdG [(5.64 ± 0.78) *vs.* (8.14 ± 0.94) ng/mL] were significantly lower than those of CT group. In the 8th week of treatment, the contents of serum SOD [(147.31 ± 16.95) *vs.* (93.20 ± 9.49) IU/mL), GSH-Px [(181.46 ± 22.39) *vs.* (117.68 ± 15.47) IU/mL] and CAT [(90.35 ± 10.35) *vs.* (54.42 ± 7.24) IU/mL] from TCMA group were significantly higher than those of CT group and the contents of serum MDA [(4.09 ± 0.59) *vs.* (8.46 ± 0.93) μmol/L], AOPP [(47.65 ± 6.76) *vs.* (91.34 ± 10.34) μmol/L] and 8-OHdG [(4.88 ± 0.69) *vs.* (7.69 ± 0.89) ng/mL] were significantly lower than those of CT group (Table 3).

3.4. Contents of chemotactic factor and adhesion molecule

In 4th week of treatment, the contents of serum MCP-1 [(213.54 ± 29.52) *vs.* (358.74 ± 41.84) pg/mL], CD40 [(147.62 ± 19.46) *vs.* (228.56 ± 31.46) pg/mL], CD40 ligand (CD40L) [(169.46 ± 18.61) *vs.* (241.35 ± 29.54) pg/mL], VE-cadherin [(4.62 ± 0.61) *vs.* (7.83 ± 0.93) μg/mL], sICAM-1 [(79.75 ± 9.14) *vs.* (126.52 ± 16.41) pg/mL] and sVCAM-1 [(57.12 ± 7.84) *vs.* (89.51 ± 10.35) pg/mL] from TCMA group were significantly lower than those of CT group. In the 8th week of treatment, the contents of serum MCP-1 [(168.62 ± 19.34) *vs.* (293.15 ± 36.32) pg/mL], CD40 [(115.64 ± 13.68) *vs.* (189.34 ± 22.56) pg/mL], CD40L [(131.42 ± 16.91) *vs.* (215.75 ± 26.75) pg/mL], VE-cadherin [(3.25 ± 0.49) *vs.* (6.03 ± 0.78) μg/mL], sICAM-1 [(56.34 ± 7.72) *vs.* (89.34 ± 9.31) pg/mL] and sVCAM-1 [(40.22 ± 5.64) *vs.* (79.24 ± 9.34) pg/mL] from TCMA group were significantly lower than those of CT group (Table 4).

Table 3
Comparison of oxidative stress parameters from two groups.

Parameters		TCM group (n = 38)	CT group (n = 31)	P
The 4th week of treatment	SOD (IU/mL)	138.54 ± 17.61	94.52 ± 10.15	< 0.05
	GSH-Px (IU/mL)	168.65 ± 15.65	103.45 ± 11.52	< 0.05
	CAT (IU/mL)	75.68 ± 9.34	48.59 ± 6.41	< 0.05
	MDA (μmol/L)	4.85 ± 0.65	8.98 ± 1.03	< 0.05
	AOPP (μmol/L)	60.34 ± 7.68	96.87 ± 11.36	< 0.05
	8-OHdG (ng/mL)	5.64 ± 0.78	8.14 ± 0.94	< 0.05
The 8th week of treatment	SOD (IU/mL)	147.31 ± 16.95	93.20 ± 9.49	< 0.05
	GSH-Px (IU/mL)	181.46 ± 22.39	117.68 ± 15.47	< 0.05
	CAT (IU/mL)	90.35 ± 10.35	54.42 ± 7.24	< 0.05
	MDA (μmol/L)	4.09 ± 0.59	8.46 ± 0.93	< 0.05
	AOPP (μmol/L)	47.65 ± 6.76	91.34 ± 10.34	< 0.05
	8-OHdG (ng/mL)	4.88 ± 0.69	7.69 ± 0.89	< 0.05

Table 4
Comparison of oxidative stress parameters from two groups.

Parameters		TCM group (n = 38)	CT group (n = 31)	P
The 4th week of treatment	MCP-1 (pg/mL)	213.54 ± 29.52	358.74 ± 41.84	< 0.05
	CD40 (pg/mL)	147.62 ± 19.46	228.56 ± 31.46	< 0.05
	CD40L (pg/mL)	169.46 ± 18.61	241.35 ± 29.54	< 0.05
	VE-cadherin (μg/mL)	4.62 ± 0.61	7.83 ± 0.93	< 0.05
	sICAM-1 (pg/mL)	79.75 ± 9.14	126.52 ± 16.41	< 0.05
	sVCAM-1 (pg/mL)	57.12 ± 7.84	89.51 ± 10.35	< 0.05
The 8th week of treatment	MCP-1 (pg/mL)	168.62 ± 19.34	293.15 ± 36.32	< 0.05
	CD40 (pg/mL)	115.64 ± 13.68	189.34 ± 22.56	< 0.05
	CD40L (pg/mL)	131.42 ± 16.91	215.75 ± 26.75	< 0.05
	VE-cadherin (μg/mL)	3.25 ± 0.49	6.03 ± 0.78	< 0.05
	sICAM-1(pg/mL)	56.34 ± 7.72	89.34 ± 9.31	< 0.05
	sVCAM-1(pg/mL)	40.22 ± 5.64	79.24 ± 9.34	< 0.05

4. Discussion

As antiplatelet drug, aspirin and clopidogrel are the common medicines used for the treatment of ischemic stroke in western medicine, which can inhibit the activation and aggregation of blood platelet so as to inhibit the formation of thrombus. They were used in the secondary prevention treatment of ischemic stroke[9,10]. However, for those patients who already had ischemic stroke, the plaque rupture of atherosclerosis in body will activate the blood platelet and improve the aggregation of blood platelet, which results in the formation of thrombus and microcirculation disturbance. The microcirculation cannot be improved barely depending on aspirin and clopidogrel to carry out the antiplatelet treatment. The neuron will be damaged due to the factors of hypoxic-ischemic and the continuous effect of secondary oxidative stress[11,12]. Salviae miltiorrhizae and ligustrazine are traditional Chinese medicine used for the treatment of cardiovascular and cerebrovascular diseases in recent years, whose effective constituents are salvianolate and ligustrazine with the effect of reducing blood viscosity, inhibiting the aggregation of blood platelet and improving microcirculation as well as scavenging oxygen free radical and reducing the oxidative stress reaction[13-16]. We carried out the adjuvant therapy with salviae miltiorrhizae and ligustrazine injection on patients with ischemic stroke in the above study. By analyzing the serum contents of markers of neural injury, we can know that the contents of serum NSE, S100B and H-FABP from TCMA group in the 4th week and the 8th week of treatment were significantly lower than those of CT group. S100B protein is a class of acid calcium-binding protein. The S100B in neuronal cells participates in the axon growth and the regulation of neuron differentiation[17,18]. NSE is a class of enolase dimer isozyme. The NSE in neuronal cells participates in the regulation of cellular energy metabolism[19]. H-FABP is one of the member of FABP. The H-FABP in neuronal cells participates in the intake of fatty acid and the regulation of oxidation power[20]. The results combined with the marker contents of neural injury indicated that the adjuvant therapy with salviae miltiorrhizae and ligustrazine can reduce the neural function injury.

The treatment value of salviae miltiorrhizae and ligustrazine injection lies in the inhibition of activation of blood platelet, the scavenging of oxygen free radical and the increasing oxidative stress reaction. In the developing process of ischemic stroke, the local tissue will produce massive oxygen free radical under the anoxic condition, which cause the oxidative stress injury on neuron cell, neurogliocyte and vascular endothelial cell. Reactive oxygen is the most important class of oxygen free radical to mediate the oxidative stress injury, which can produce oxidation reaction with various components of cells leading to an injury of cellular structure and function. The product of the oxidation reaction between the lipid components of organelle membrane in cytoplasm and cytomembrane is MDA. The outcome of oxidation reaction occurred in the protein components of endochylema is AOPP. The outcome of oxidation reaction in the nucleic acid of cell nucleus is 8-OHdG[21,22]. Salviae miltiorrhizae and ligustrazine have an direct effect on scavenging the oxygen free radical produced in the process of oxidative stress reaction. After the oxygen free radical is scavenged, the oxidized lipid, protein and nucleic acid will be reduced correspondingly. The analysis of the oxidative product contents in serum from two groups proved that the contents of serum MDA, AOPP and 8-OHdG from TCMA group were significantly lower than those of CT group, which indicated that the adjuvant therapy with salviae miltiorrhizae and ligustrazine can scavenge the oxygen free radical overproduced in the developing process of ischemic stroke and alleviate the oxidative stress reaction. SOD, GSH-Px and CAT are the important antioxidant enzymes in the body, which can scavenge the oxygen free radical produced in the process of oxidative stress reaction through redox reaction and keep the local tissue in reducing state[23]. When the oxidative stress reaction is in excessive activation and the oxygen free radical is produced massively, the antioxidant enzymes will be constantly consumed. We have found from the analysis of contents of antioxidant enzymes mentioned above, that the contents of serum SOD, GSH-Px and CAT from TCMA group were significantly higher than those of CT group.

The main thrombus composition of intracranial artery in patients with ischemic stroke is blood platelet. The vital pathological links of cerebral infarction lie in the rupture of atheromatous plaque, aggregation of blood platelet at the part of plaque rupture, the activation of blood platelet and the formation of thrombus. Chemotactic factor and adhesion molecule are the important molecules for mediating the blood platelet to move and adhere towards the endarterium and plaque. MCP-1, CD40 and CD40L are chemotactic factors to mediate the directional movement of cells, which infiltrate the inflammatory cells such as monocyte and granulocyte through the endarterium and plaque, improve the local release of protease by inflammatory cells in plaque, change the plaque feature and induce the plaque rupture[24,25]. The analysis of contents of chemotactic factors in serum from two groups proved that the contents of serum MCP-1, CD40 and CD40L from TCMA group were significantly lower than those of CT group. VE-cadherin, ICAM-1 and VCAM-1 are the cytokines to mediate the adhesion amang cells or cells and tissues, which can improve the mutual adhesion of blood platelet with endarterium and plaque, and simultaneously improve the mutual adhesion between leukocyte and vascular endothelium which form aggregation with blood platelet, thus promoting the formation of thrombus[26]. The analysis of the contents of adhesion molecules in serum from two groups proved that the contents of serum VE-cadherin, ICAM-1 and VCAM-1 from TCMA group were significantly lower than those of CT group, which indicated that the adjuvant therapy with salviae miltiorrhizae and ligustrazine can inhibit the generation of chemotactic factors and adhesion molecules in the developing process of ischemic stroke.

In conclusion, adjuvant therapy with salviae miltiorrhizae and

ligustrazine hydrochloride injection can reduce the neural function injury in patients with ischemic stroke, inhibit the oxidative stress reaction and the generation of chemotactic factors and adhesion molecules, which is an effective medicine for treating ischemic stroke.

Conflict of interest statement

The authors report no conflict of interest.

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