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Effect of PAS triple therapy on nerve injury, oxidative stress and inflammatory response in patients with cerebral infarction

Li−Jun Deng✉

Neurology Department, Affiliated Hospital of Jianghan University in Hubei Province, Wuhan City, Hubei Province, 430015

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ABSTRACT

Objective: To study the effect of probucol + aspirin + atorvastatin (PAS) triple therapy on nerve injury, oxidative stress and inflammatory response in patients with cerebral infarction. **Methods:** Patients with acute cerebral infarction who were treated in Affiliated Hospital of Jianghan University between February 2015 and January 2015 were selected and randomly divided into the PAS group who received probucol + aspirin + atorvastatin triple therapy and the control group who received aspirin + atorvastatin double therapy. The markers of nerve injury, oxidative stress and inflammatory response were determined before treatment and 15 d after treatment. **Results:** 15 d after treatment, peripheral blood Keap-1 expression and serum GPX1 contents of both groups of patients were significantly higher than those before treatment while peripheral blood Nrf-2 and ARE expression as well as serum S100B, NSE, sTRAIL, FKN, HMGB-1, sICAM-1, Chemerin and 8-iso-PGF2α contents were significantly lower than those before treatment, and peripheral blood Keap-1 expression and serum GPX1 content of PAS group were significantly higher than those of control group while peripheral blood Nrf-2 and ARE expression as well as serum S100B, NSE, sTRAIL, FKN, HMGB-1, sICAM-1, Chemerin and 8-iso-PGF2α contents were significantly lower than those of control group. **Conclusion:** PAS triple therapy can reduce the nerve injury as well as oxidative stress response and inflammatory response in patients with cerebral infarction.

1. Introduction

Acute cerebral infarction is a common clinical cerebrovascular disease with rising morbidity as well as high disability rate and mortality rate. Carotid plaque is an independent risk factor for cerebral infarction, and the unstable plaque rupture, platelet activation as well as thrombus formation and falling off are important mechanisms of acute cerebral infarction[1,2]. In clinical practice, stabilizing plaques and inhibiting platelet activation on the basis of neurotrophy and protection drug therapy helps prevent the further expansion of infarction scope and reduce the ischemia hypoxia of brain tissue[3]. Aspirin and atorvastatin are the common drugs for antiplatelet and stabilizing atheromatous plaques respectively, and they have been widely used for the clinical treatment of cerebral infarction[4]. Probucol is a new type of lipid-lowering drug, which not only has the effects of regulating blood lipid metabolism and stabilizing atheromatous plaque, but can also inhibit the oxidative stress and inflammation damage to the brain tissue in the process of ischemia hypoxia[5]. The PAS triple therapy of probucol + aspirin + atorvastatin has been gradually used in the treatment of cerebral infarction, but there is no report about the changes of oxidative stress and inflammatory response after triple therapy. The effect of PAS triple therapy on nerve injury, oxidative stress and inflammatory response in patients with cerebral infarction was analyzed in the following study.

2. Research subject information and research methods

2.1 Clinical information of research subjects

Patients with acute cerebral infarction who were treated in Affiliated Hospital of Jianghan University between February 2015

✉Corresponding author: Li-Jun Deng, Neurology Department, Affiliated Hospital of Jianghan University in Hubei Province, Wuhan City, Hubei Province, 430015.
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and January 2015 were selected, all patients were diagnosed with cerebral infarction by cranial imaging examination, and the patients with autoimmune diseases and malignant tumors and the patients with history of infection and lipid-lowering drug taking within the past 4 weeks were excluded. A total of 116 patients were enrolled in the study, and random number table was used to divide the patients into two groups, each with 58 cases. PAS group included 32 male cases and 26 female cases that were 42-63 years old; control group included 34 male cases and 24 female cases that were 41-65 years old. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Therapy

Both groups of patients received nutritional support, neuroprotection, anti-free radical and other conventional treatments, patients with history of hypertension received antihypertensive drugs, and patients with history of diabetes received insulin to control blood sugar. Control group received aspirin + atorvastatin treatment on this basis, and the method was as follows: Aspirin Enteric-coated Tablets 100 mg, taken orally, 1/d as well as atorvastatin calcium tablet 20 mg, taken orally, 1/d; PAS group received probucol + aspirin + atorvastatin therapy on the basis, and the methods were as follows: probucol tablets 0.25 g, taken orally, 2 times/d, and same Aspirin Enteric-coated Tablets and atorvastatin calcium tablet usage as those of control group.

2.3 Serum molecule content detecting

Before treatment and 15 d after treatment, 3 mL of cubital venous blood was collected from two groups of patients and centrifuged to separate serum, and then enzyme-linked immunosorbent assay kit was used to determine S100B, NSE, sTRAIL, 8-iso-PGF2α, GPX1, FKN, HMGB-1, SICAM-1 and Chemerin levels.

2.4 Peripheral blood molecule expression detecting

Before treatment and 15 d after treatment, 1-2 mL of cubital venous blood was collected from two groups of patients, whole blood RNA extraction kit was used to separate RNA and synthesize it into cDNA by reverse transcription, the primers for Keap-1, Nrf-2 and ARE were designed, fluorescence quantitative PCR amplification was conducted, and the amplification curve was referred to calculate the Keap-1, Nrf-2 and ARE mRNA expression.

2.5 Statistical methods

SPSS 19.0 software was used to input data, the differences in measurement data between two groups were analyzed by t test and P <0.05 indicated statistical significance in differences in test results.

3. Results

3.1 Nerve cell injury markers

Before treatment and 15 d after treatment, analysis of serum nerve cell injury markers S100B (pg/mL), NSE (pg/mL) and sTRAIL (ng/mL) contents between two groups of patients was as follows: before treatment, serum S100B, NSE and sTRAIL contents were not significantly different between two groups of patients; 15d after treatment, serum S100B, NSE and sTRAIL contents of both groups of patients were significantly lower than those before treatment, and serum S100B, NSE and sTRAIL contents of PAS group were significantly lower than those of control group.

Table 1.

Changes in nerve cell injury markers before and after treatment.

Groups	n	Time	NSE	S100B	sTRAIL
PAS group	58	Before treatment	23.42±3.41	2.18±0.29	93.51±10.38
		15 d after treatment	7.79±0.94*#	0.84±0.11*#	37.69±4.13*#
Control group	58	Before treatment	23.19±3.28	2.23±0.27	94.41±10.22
		15 d after treatment	12.18±1.48#	1.29±0.15#	54.28±6.72#

*: comparison between PAS group and control group after treatment, P< 0.05; #: comparison within group between before treatment and 15d after treatment, P<0.05.

3.2 Oxidative stress markers

Before treatment and 15 d after treatment, analysis of oxidative stress markers Keap-1, Nrf-2 and ARE in peripheral blood as well as oxidative stress markers 8-iso-PGF2α (pg/mL) and GPX1 (U/L) in serum between two groups of patients was as follows: before treatment, peripheral blood Keap-1, Nrf-2 and ARE expression as well as serum 8-iso-PGF2α and GPX1 contents were not significantly different between two groups of patients; 15 d after treatment, peripheral blood Keap-1 expression and serum GPX1 contents of both groups of patients were significantly higher than those before treatment while peripheral blood Nrf-2 and ARE expression as well as serum 8-iso-PGF2α contents were significantly lower than those before treatment, and peripheral blood Keap-1 expression and serum GPX1 content of PAS group were significantly higher than those of control group while peripheral blood Nrf-2 and ARE expression as well as serum 8-iso-PGF2α content were significantly lower than those of control group.

Table 2.

Changes in oxidative stress markers before and after treatment.

Groups	n	Time	Peripheral blood			Serum	
			Keap-1	Nrf-2	ARE	GPX1	8-iso-PGF2α
PAS group	58	Before treatment	1.02±0.14	0.99±0.13	1.04±0.15	32.58±5.62	582.3±72.3
		15 d after treatment	2.52±0.34*#	0.32±0.05*#	0.28±0.04*#	71.31±9.34*#	241.2±33.8*#
Control group	58	Before treatment	1.01±0.13	1.02±0.15	1.02±0.17	33.12±4.59	585.2±69.3
		15 d after treatment	1.77±0.24#	0.71±0.09#	0.58±0.09#	53.28±7.59#	378.5±52.3#

*: comparison between PAS group and control group after treatment, P<0.05; #: comparison within group between before treatment and 15d after treatment, P <0.05.

Table 3.

Changes in inflammation markers before and after treatment.

Groups	*n*	Time	FKN	HMGB-1	sICAM-1	Chemerin
PAS group	58	Before treatment	108.52±13.57	41.93±5.59	313.52±33.95	142.31±16.93
		15 d after treatment	62.13±7.79*#	20.38±3.82*#	170.49±20.32*#	72.34±9.86*#
Control group	58	Before treatment	110.13±13.85	42.31±5.69	315.68±36.86	144.14±15.74
		15 d after treatment	83.28±9.38#	29.38±4.28#	227.95±32.93#	102.46±13.82#

*: comparison between PAS group and control group after treatment, $P<0.05$; #: comparison within group between before treatment and 15 d after treatment, $P<0.05$.

3.3 Inflammation markers

Before treatment and 15 d after treatment, analysis of serum inflammation markers FKN (ng/mL), HMGB-1 (pg/mL), sICAM-1 (ng/mL) and Chemerin (ng/mL) contents between two groups of patients was as follows: before treatment, serum FKN, HMGB-1, sICAM-1 and Chemerin contents were not significantly different between two groups of patients; 15 d after treatment, serum FKN, HMGB-1, sICAM-1 and Chemerin contents of both groups of patients were significantly lower than those before treatment, and serum FKN, HMGB-1, sICAM-1 and Chemerin contents of PAS group were significantly lower than those of control group.

4. Discussion

Acute cerebral infarction is a common clinical disabling and deadly disease, atherosclerosis is the pathological basis of cerebral infarction, and plaque stability reduction and rupture, platelet activation and thrombosis are the key links in the process of vascular infarction. In clinical practice, antiplatelet by aspirin and plaque stabilization by atorvastatin are important means in the treatment of cerebral infarction and can avoid further thrombosis and ischemic hypoxic injury aggravation. Probucol is a new type of lipid-lowering drug that can promote the expression of apolipoprotein E to regulate the transfer of cholesterol and delay the process of atherosclerosis[6]. In recent years, research on probucol has confirmed that the drug can not only regulate lipid metabolism, but also promote the scavenging of inflammatory mediators and oxygen free radicals, and reduce inflammation and oxidative stress damage to nerve function; in addition, probucol can also increase the activity of various antioxidant enzymes and enhance the ability of local tissues to withstand oxidative damage[7]. It has been reported that the PAS triple therapy on the basis of plaque stabilization by atorvastatin and antiplatelet by aspirin can improve the neural function in patients with acute cerebral infarction, but there is no report about the changes of oxidative stress and inflammation in patients with cerebral infarction.

After the occurrence of cerebral infarction, brain tissue injury caused by ischemia hypoxia can cause the neurons and glial cells in local tissue to rupture, and the corresponding molecules in cells are released into the extracellular area and enter the blood circulation through the blood brain barrier. NSE and S100B are the markers of neurons and glial cells respectively, the former participates in glucose catabolism and energy supply in neurons, the latter is involved in the formation of glial cell skeleton, and the rupture of neurons and glial cells will cause serum NSE and S100B levels to rise[8,9]. TRAIL is a molecule that promotes apoptosis to causes nerve cell damage in ischemic hypoxic state, and sTRAIL is its soluble form and can reflect the degree of apoptosis damage[10]. In order to define the effect of PAS triple therapy on the nerve damage in patients with acute cerebral infarction, and the changes in serum levels of these nerve damage markers before and after treatment were analyzed in the study, and the results showed that serum S100B, NSE and sTRAIL contents of both groups of patients significantly decreased after treatment, and serum S100B, NSE and sTRAIL contents of PAS group after treatment were significantly lower than those of control group. This means that both atorvastatin + aspirin double therapy and PAS triple therapy can relieve the nerve injury in patients with cerebral infarction, and PAS triple therapy is better than double therapy in reducing the nerve injury.

Massive oxygen free radical generation and oxidative stress response activation are the important pathological links in the nerve injury under hypoxic hypoxia condition[11,12]. Keap1-/Nrf2/ARE is an important antioxidant pathway within cells, the continuous generation of oxygen free radicals can inhibit the Keap-1 expression and cause Keap-1 to be dissociated with Nrf-2, and the free Nrf-2 enters the nucleus to identify the ARE sequence of a variety of antioxidant genes and start the gene expression[13,14]. GPX1 is one of the antioxidant genes regulated by Nrf-2, and the product encoded by it has the effect of scavenging free radicals. The damaging effect of massively generated oxygen free radicals in the process of cerebral infarction is associated with the lipid peroxidation in cellular structure, and 8-iso-PGF2α is the product of lipid oxidation, and can reflect the generation of free radicals and the degree of oxidative stress injury. In the study, analysis of the changes in these oxidative stress markers before and after treatment indicated that peripheral blood Keap-1 expression and serum GPX1 contents of both groups of patients significantly increased while peripheral blood Nrf-2 and ARE expression as well as serum 8-iso-PGF2α contents significantly decreased after treatment, and peripheral blood Keap-1 expression and serum GPX1 content of PAS group after treatment were significantly higher than those of control group while peripheral blood Nrf-2 and ARE expression as well as serum 8-iso-PGF2α content were significantly lower than those of control

group. This means that both atorvastatin + aspirin double therapy and PAS triple therapy can relieve the oxidative stress reaction in patients with cerebral infarction, and PAS triple therapy is better than double therapy in reducing the oxidative stress.

Inflammatory response is the pathological change throughout the course of atherosclerosis and cerebral infarction, and many cytokines are closely related to inflammatory response. KFN is the adhesion molecule in CX3C family, ICAM-1 is an intercellular adhesion molecule, and both can promote the adhesion of lymphocytes, mononuclear macrophages and other inflammatory cells with vascular endothelial cell adhesion, and accelerate the formation of endangium foam cells and the development of atheromatous plaque[15,16]; HMGB-1 is a member of the high mobility group box family, which is expressed in both vascular endothelial cells and smooth muscle cells, can locally recruit a variety of inflammatory cells and increase the release of inflammatory mediators, and is not only involved in the process of atherosclerosis, but also associated with the ischemic hypoxic injury of brain tissue[15,16]; Chemerin is a kind of adipocytokine with chemotaxis effect, which can promote the infiltration of local inflammatory cells and inflammatory mediators in local atheromatous plaque and local cerebral infarction so as to accelerate the atherosclerosis process and increase the degree of cerebral infarction[17]. In the study, analysis of the changes in these inflammatory response markers before and after treatment indicated that serum FKN, HMGB-1, sICAM-1 and Chemerin contents of both groups of patients significantly decreased after treatment, and serum FKN, HMGB-1, sICAM-1 and Chemerin contents of PAS group after traetment were significantly lower than those of control group. This means that both atorvastatin + aspirin double therapy and PAS triple therapy can reduce the inflammation in patients with cerebral infarction, and PAS triple therapy is better than double therapy in reducing the inflammatory reaction.

The value of probucol + aspirin + atorvastatin triple therapy for cerebral infarction was mainly analyzed in the study, and the preliminary conclusions are as follows: PAS triple therapy is better than double therapy in reducing the nerve injury as well as oxidative stress response and inflammatory response in patients with cerebral infarction.

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