

Original Article

Effects of persistent oxidative stress on the treatment of acute cerebral infarctions

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Abstract: The objective of this study is to explore the effects of persistent oxidative stress on the treatment of acute cerebral infarction (ACI). Sixty patients with ACI were assigned to the test group and 30 patients were included as healthy controls. Venous blood was collected from the patients and controls before treatment and 24, 48, and 72 h following the treatment as part of the medical examination. Alterations in the levels of superoxide dismutase (SOD), lipid peroxide (LPO), and nitric oxide (NO) were detected. The control group had a comparatively higher SOD level, which gradually decreased with the severity of cerebral infarction (CI) and as LPO and NO levels also declined. In contrast, LPO and NO levels increase with the exacerbation of CI. Patients experiencing severe oxidative stress recovered more slowly. Following treatment, patients with mild CI recovered the quickest, whereas patients with severe CI were the slowest to recover. A statistically significant difference in ADL scores was observed between groups. The severity of CI is positively correlated with the degree of oxidative stress, i.e., higher levels of oxidative stress lead to a slower recovery of patients following treatment.

Keywords: Cerebral infarction, oxidative stress, acute phase, effect

Introduction

CI results mainly from atherosclerosis and thrombosis of the arteries that supply blood to the brain, causing luminal stenosis or occasionally occlusions and inducing acute cerebral vascular insufficiency. In addition, CI partly stems from the ability solids, liquids, or gas to enter the cerebral or carotid arteries supplying cerebral circulation through the blood stream, blocking or reducing blood flow and causing necrosis of the nervous tissue. CI is the most common cerebrovascular disease (CVD), accounting for 75% of all cases, with a high mortality (10-15%), disability, and recurrence rate. CI is also correlated with significantly higher mortality due to recurrent stroke [1].

In most cases, CI is induced by cerebral atherosclerosis and thrombosis. Studies have demonstrated that oxygen free radicals (OFR) are involved in fatty streak formation, plaque progression, and atherosclerotic plaque ruptures, indicating the role of oxidative stress in CI pathogenesis [2, 3].

Oxidative stress is defined as a disturbance in the balance between the production of highly reactive molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Under oxidative stress, the endogenous antioxidant defenses become overactive, ultimately resulting in tissue damage [4]. The generation of free radicals is in a dynamic equilibrium with their removal in healthy individuals, and is found to pathologically increase in patients with acute cerebral infarction (ACI), inducing ischemic cell necrosis, and causing aggregations to occur with ACI [5, 6]. In the present study, the effects of persistent oxidative stress on the efficacy of ACI treatments were analyzed.

Subjects and methods

General data

The following individuals were included in the test group: 60 ACI patients, comprising 40 males and 20 females between the ages of 41 and 83 years with a median age of 66.37 ± 8.45 years, were selected from patients in the

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Table 1. Comparison of clinical data between two groups

Items	Test group	Control group	P value
Male/Female (cases)	40/20	15/15	> 0.05
Age (years old)	66.37 ± 8.45	65.62 ± 9.41	> 0.05
Systolic pressure (mmHg)	143.26 ± 14.77	135.47 ± 12.31	< 0.05
Diastolic pressure (mmHg)	87.30 ± 12.36	81.45 ± 8.80	< 0.05
Fasting blood-glucose (mg/dl)	106.32 ± 41.32	100.31 ± 21.70	> 0.05
Triglyceride (mg/dl)	165.55 ± 93.38	138.37 ± 73.07	> 0.05

Department of Neurology of our hospital between March 2011 and March 2012. All patients were selected with the diagnostic criteria for atherosclerotic CI proposed in the 1995 4th National Conference on Cerebrovascular Disease, which was confirmed by brain CT or MRI within 2 days of the first attack. The following exclusion criteria were used: 1) Patients with cerebral hemorrhage, unconsciousness, malignant tumor, severe malnutrition, epilepsy, peripheral angiopathy, etc.; 2) Patients with coronary heart disease, cardiac insufficiency, diabetes, gout, and severe lung, liver, or kidney diseases; 3) Patients who had recently taken vitamin C, vitamin E, and edaravone. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Dagang Oil Field General Hospital. Written informed consent was obtained from all participants.

The following individuals were included in the control group: 30 healthy controls, comprising 15 males and 15 females between the ages of 40 and 80 years with a median age of 65.62 ± 9.41 years, were recruited from the Medical Examination Center during the corresponding time. CVD was excluded after detailed interrogation and examination.

Sample collection

Venous blood (2 mL) was collected from the ACI patients and healthy controls before treatment and 24, 48, and 72 h following treatment during a follow-up medical examination. Samples were stored in tubes containing ethylene diamine tetraacetic acid (EDTA) and were centrifuged at 3000 rpm for 15 min. The supernatant was collected and stored at -80°C for further use.

Illness assessment

Neurologic impairment of the ACI patients was scored on admission according to the National

Institutes of Health Stroke Scale (NIHSS) score, which was used to divide our patients into the following three groups based on severity: 19 cases in the mild group (NIHSS < 4), 24 cases in the moderate group (4 ≤ NIHSS ≤ 15), and 17 cases in the severe group (NIHSS > 15).

For the Ability of Daily Living (ADL) score, a score of 100 indicates full function and a score higher than 60 indicates that patients are essentially independent. A score ranging from 40 to 61 indicates that the patients need some assistance. A score between 20 and 40 indicates that patients are mostly dependent. A score of less than 20 indicates that patients are fully dependent.

Observational index and method

An enzyme-linked immunosorbent assay (ELISA) was performed according to the protocol from the kit (SIGMA Corporation, St. Louis, MO) to detect the activity of superoxide dismutase (SOD) and lipid peroxide (LPO). Colorimetric and spectrophotometric analyses were performed to measure nitric oxide (NO) levels.

Statistical analysis

Qualitative data analysis was performed using the chi square test. For quantitative comparisons, normally distributed data were analyzed with a *t*-test, and data with abnormal distribution were analyzed with the Mann-Whitney U test. Any contributions from gender were analyzed with a chi square test. Other clinically relevant factors, including age, blood pressure, FBG, triglyceride, and the changes of SOD, LPO, and NO, were analyzed with *t*-test comparisons. The differences between ADL scores before and after treatment were analyzed with a Mann-Whitney U test. All data were analyzed with SPSS19.0, and *P* < 0.05 was considered a significant difference.

Results

Clinical data

Of all clinical indicators analyzed, only blood pressure (*P* < 0.05) showed a significant difference (**Table 1**).

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Table 2. Comparison of SOD level in patients (u/g.Hb)

	0 h	24 h	48 h	72 h
Control group	2156.49 ± 219.45	-	-	-
Mild group	1703.50 ± 73.44	1857.00 ± 68.04	1937.10 ± 65.28	2000.19 ± 63.62
Moderate group	1522.36 ± 60.73	1600.32 ± 63.00	1702.61 ± 57.62	1811.07 ± 47.65
Severe group	1269.80 ± 49.05	1320.14 ± 45.93	1408.52 ± 40.04	1633.58 ± 47.71
P value	< 0.001	< 0.001	< 0.001	< 0.001

Table 3. Comparison of LPO level in patients (umol/L)

	0 h	24 h	48 h	72 h
Control group	10.88 ± 4.18	-	-	-
Mild group	12.04 ± 1.69	11.82 ± 1.33	11.41 ± 1.07	10.92 ± 1.09
Moderate group	14.38 ± 3.19	13.50 ± 1.71	13.04 ± 1.25	12.30 ± 1.30
Severe group	16.32 ± 5.52	15.90 ± 1.47	15.27 ± 1.17	14.31 ± 1.55
P value	< 0.001	< 0.001	< 0.001	< 0.001

Discussion

CVD is the most prevalent disease affecting human health, having a high morbidity, mortality, and disability rates associated with it. CI is the generic term for ischemic stroke, which includes cerebral thrombosis, lacunar

infarction, and cerebral embolism. Cerebral embolism is characterized by cerebral lesions induced by disturbances in the blood supply to the brain, and it accounts for 70% of all stroke cases. CI, with a disability rate of up to 50% following an event, is globally second only to death from coronary heart disease. China alone has the second highest CI incidence in the world [7]. Common causes for stroke include atherosclerosis, [8] complications of hypertension, diabetes, hyperlipidemia, arteritis, polycythemia, thrombocytopenia, thrombotic thrombocytopenic purpura, cerebral amyloid angiopathy, Moyamoya disease, fibromuscular dysplasia, aortic dissection, cardiogenic and unknown emboli, and antiphospholipid syndrome.

Oxidative stress is defined as a disturbance in the balance between the production of highly reactive molecules, such as ROS and RNS, and the antioxidant defenses that occur under harmful stimulation, ultimately resulting in tissue damage [9]. Interruptions to cerebral blood flow cause ischemia, which can induce brain cell necrosis even if ischemia occurs for only a few minutes. Oxidative stress resulting from ischemia/reperfusion (I/R) plays a critical role in causing irreversible tissue damage [10-14], where the free radical production sharply increases and oxidative stress is substantially elevated following CI [10]. Free radicals directly damage cells, causing the peroxidation of lipids, proteins, and nucleic acids, resulting in membrane destabilization, protein degradation, DNA strand breakage, hyaluronic acid

Change in SOD level in patients

The control group showed relatively higher SOD levels, which gradually declined as the severity of CI increased. The difference between the control and treatment groups was significant ($P < 0.001$). Following treatment, SOD levels were restored to baseline levels (**Table 2**).

Change in LPO level in patients

The control group expressed comparatively lower LPO levels that increased with CI severity. The difference between the control and treatment groups was significant ($P < 0.001$). Following treatment, LPO levels were reduced (**Table 3**).

Change in NO level in patients

NO levels were relatively lower in the control group, which increased with the severity of CI. The difference between two groups was significant ($P < 0.001$). Following treatment, NO levels were reduced (**Table 4**).

Comparison of ADL score before and after the treatment

After treatment, the patients belonging to the mild group recovered the quickest, whereas patients in the severe group were the slowest to recover. Statistically significant differences in ADL scores were observed between these groups (**Table 5**).

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Table 4. Comparison of NO level in patients (nmol/L)

	0 h	24 h	48 h	72 h
Control group	338.45 ± 29.67	-	-	-
Mild group	457.46 ± 35.07	428.33 ± 31.09	405.17 ± 30.85	360.14 ± 23.57
Moderate group	549.50 ± 46.99	500.74 ± 27.56	460.48 ± 25.48	412.00 ± 25.07
Severe group	689.83 ± 70.51	647.35 ± 32.49	601.08 ± 30.22	551.78 ± 27.36
P value	< 0.001	< 0.001	< 0.001	< 0.001

Table 5. Comparison of ADL score before and after the treatment

	Before treatment	1 w after treatment	4 w after treatment	6 w after treatment
Mild group	41.55 ± 14.74	50.61 ± 18.24	66.73 ± 13.92	78.30 ± 18.27
Moderate group	36.24 ± 18.35	42.32 ± 14.72	57.29 ± 13.05	68.64 ± 15.83
Severe group	31.60 ± 12.90	35.10 ± 12.57	40.33 ± 12.06	54.46 ± 16.80
P value	< 0.001	< 0.001	< 0.001	< 0.001

depolymerization, cell collapse, and the promotion of irreversible neuronal death [10, 11]. Ciancarelli and colleagues [15] demonstrated that a reduction in the redox imbalance could accelerate the recovery of neural function in patients following stroke. Studies have shown that oxidative stress also promotes the activation of matrix metalloproteinase-9, promoting damage to the blood-brain barrier and increasing the infarct area. During ACI, production of free radicals pathologically aggravates oxidative stress causing I/R injury of neurons, whereas plasma antioxidant reactions can protect neurons.

NO is synthesized by NO synthase (NOS) that abundantly exists in the nervous system as an isozyme. It is composed of three subtypes, neuronal NOS (nNOS), which is expressed in the nervous system, endothelial NOS (eNOS), which is expressed under normal conditions, and inducible NOS (iNOS), which is expressed following injury. iNOS and nNOS are mainly expressed in neurons, neurogliaocytes, and vascular muscle cells [16-18]. Several studies [19] have demonstrated that endothelial cells release NO to maintain cerebral perfusion following ischemia. However, studies with iNOS and nNOS knockout mice have demonstrated that the activation of iNOS and nNOS is harmful to brain cells after ischemia [20, 21]. In the present study, the control group exhibited a relatively low NO concentration, which increased as CI increased in severity. The difference between two groups was significant ($P < 0.001$). NO levels declined after the treat-

ment, indicating that high levels of NO may further aggravate brain damage.

SOD, an acidic metalloenzyme that is common to many organisms, is categorized into the following three subtypes in humans according to the associated metal ion: Cu, Zn-SOD, found in the cytoplasm, Mn-SOD, found in the mitochondria, and EC-SOD, found in the extracellular space. SOD is an important member of the antioxidant system, and is a significant scavenger of superoxide anions, which reduce or inhibit lipid peroxidation, deferring senescence and preventing damage to biological macromolecules. Experiments have proven that exogenous SOD can ameliorate I/R injury [22]. Huang [23] also demonstrated that Mn-SOD is an effective therapeutic target in the treatment of ischemic stroke because of its anti-oxidative effects and ability to regulate oxidative stress. SOD levels were found to be relatively high in the control group, which was found to gradually decrease with increase in CI severity. The difference between the control and therapeutic groups was significant ($P < 0.001$). SOD levels increased following treatment, consistent with the results obtained from previous studies.

LPO is the product of membrane lipid peroxidation of polyunsaturated fatty acids of cell membranes by oxygen free radicals, which impair membrane structure and cell function, resulting in multiple diseases [24]. In the present study, the control group exhibited comparatively lower LPO level, which increased according to CI severity. The difference between the control

and treatment groups was statistically significant ($P < 0.001$). LPO levels declined following the treatment. Patients in the mild group were observed to recover the quickest, whereas patients in the severe group recovered the slowest. A statistically significant difference in ADL scores was observed between the control and treatment groups.

In conclusion, the severity of CI is positively correlated with the degree of oxidative stress, i.e., a higher level of oxidative stress leads to slower recovery in patients following CI treatment.

Disclosure of conflict of interest

None.

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