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# The effects of large doses of vitamin C and vitamin E on nerve injury, neurotrophic and oxidative stress in patients with acute craniocerebral injury

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ABSTRACT

**Objective**: To study the effects of large doses of vitamin C and vitamin E on nerve injury, neurotrophic and oxidative stress in patients with acute craniocerebral injury. **Methods:** Patients with acute craniocerebral trauma who were admitted to the Third People's Hospital of Zigong from April 2014 to December 2016 were selected as the subjects and were randomly divided into two groups. The control group received conventional treatment, and the intervention group received large doses of vitamin C and vitamin E combined with conventional treatment. On the 3$^{th}$ day and 7$^{th}$ day after treatment, peripheral blood was collected and serum was isolated, then the contents of nerve injury index NSE, S100B, NGB, UCH-L1, Tf, Ft and neurotrophic indexes NTF-$\alpha$, BDNF, NGF and IGF-I were determined by Enzyme-linked immunosorbent assay kit, and the contents of SOD, GPx, CAT, $OH^-$, $O_2^-$, MDA and AOPP were measured by radioactive immunoprecipitation kit. **Results**: 3$^{th}$ day and 7$^{th}$ day after treatment, the contents of NSE, S100B, NGB, UCH-L1, Tf, Ft, NTF-$\alpha$, BDNF, NGF, IGF-I, $OH^-$, $O_2^-$, MDA and AOPP in the intervention group were all significantly lower than those in the control group. The content of SOD, GPx and CAT in serum in the intervention group was significantly higher than that in the control group. **Conclusions**: High-dose vitamin C and vitamin E treatment can alleviate nerve injury, oxidative stress response, and improve neurotrophic state in patients with acute craniocerebral injury.

## 1. Introduction

Acute craniocerebral injury is a common traumatic disease in brain surgery. After the injury by direct physical and indirect chemical factors, neurons and glial cells in the nervous system will be affected by sustained damage, and nerve function irreversible damage happens[1-3]. In the treatment of acute craniocerebral injury, decompressive craniectomy, mannitol dehydration and other methods can effectively remove direct physical injury factors. While

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neuroprotective agents, antioxidants and other drugs can relieve the indirect chemical injury to some extent, but the effect is not ideal. Hyperactivation of oxidative stress is an important factor of indirect chemical injury in acute craniocerebral injury. It is characterized by the continuous mass production of oxygen free radicals and oxidative reactions with lipids and proteins in the brain tissue, causing tissue damage and destruction. Based on the effect of hyperactivation of oxidative in the development of craniocerebral injury, scavenging oxygen free radicals is an important method to treat craniocerebral injury[4]. Vitamin C and vitamin E are vitamins that have antioxidant activity and are used in antioxidant therapy[5]. In the following studies, we specifically analyzed the effects of large doses of vitamin C and vitamin E on nerve injury, neurotrophic and oxidative stress in patients with acute craniocerebral injury.

## 2. Materials and methods

### 2.1. Information of patients

Patients with acute craniocerebral trauma who were admitted to the Third People's Hospital of Zigong from April 2014 to December 2016 were selected as the subjects. All patients had a clear history of trauma, the Glasgow coma scale (GCS) were 3-12 points, and were confirmed by head CT examination as brain injury. Patients with a history of craniocerebral injury, stroke, and brain tumor were excluded. A total of 84 patients were enrolled in the group. After obtaining the informed consent of the patients and the approval of the hospital ethics committee, the enrolled patients were randomly divided into two groups, each with 42 cases. There were 28 males and 14 females in the intervention group, including 27 car accident injuries, 11 fall injures and 4 strikes injures, and the age was 25 to 49 years old, while control group has 29 males and 13 females, including 28 car accident injuries, 9 fall injures and 5 strikes injures, and the age was 25 to 49 years old.

### 2.2. Therapeutic method

After admission, both groups were treated with routine descending cranial pressure by dehydration, anti-infection with antibiotics, stomach protection with proton pump inhibitors, hemostasis with thrombin, and scavenging oxygen free radical with edaravone injection. Patients with surgical indications are treated with acute hematoma removal. Patients in the intervention group were given a large dose of vitamin C and vitamin E on the basis of the above routine treatment, and the methods is as following: $1^{th}$-$4^{th}$ day, Vitamin C 4.0 g, intravenous drip, 2 times a day; $5^{th}$-$7^{th}$ day, vitamin C 3.0 g, intravenous drip, 2 times a day; Vitamin E 100 mg, muscle injection, 1 time a day were given for the first 7 days.

### 2.3. Examination index

Between $4^{th}$ day and $7^{th}$ day after treatment, the elbow vein blood of the two groups was collected 5-8 mL. After the serum was obtained by centrifugation, the contents of NSE, S100B, NGB, UCH-L1, Tf, Tf, NTF-$\alpha$, BDNF, NGF, and IGF-I were determined by using enzyme-associated immunosorbent assay (Elisa) kit.

### 2.4. Statistical method

SPSS19.0 software was used to input and analyze data, and the measurement data was expressed in the form of mean ± standard deviation, and *t*-test analysis was used. The counting data is expressed in frequency form and analyzed by chi-square test. $P < 0.05$ was considered as the criterion of statistical significance.

## 3. Results

### 3.1. Index of nerve injury

On the $4^{th}$ day and $7^{th}$ day after treatment, the contents of NSE, S100B, NGB, UCH-L1, Tf and Ft were determined in the serum of the two groups by Elisa kit. The contents of NSE, S100B, NGB, UCH-L1, Tf and Ft in the serum of the intervention group were significantly lower than those in the control group. The differences of NSE, S100B, NGB, UCH-L1, Tf and Ft in serum of the two groups were statistically significant on $4^{th}$ day and $7^{th}$ day after treatment(Table 1).

**Table 1**
The comparison of the nerve injury index in the serum after treatment.

| Index | Time | Intervention group | Control group | *P* |
|---|---|---|---|---|
| NSE (ng/mL) | $4^{th}$ day | 27.41±3.95 | 44.19±7.37 | <0.05 |
| | $7^{th}$ day | 19.38±2.52 | 28.51±4.12 | <0.05 |
| S100B (ng/mL) | $4^{th}$ day | 1.03±0.18 | 1.83±0.25 | <0.05 |
| | $7^{th}$ day | 0.78±0.10 | 1.30±0.18 | <0.05 |
| NGB (μg/mL) | $4^{th}$ day | 1.85±0.22 | 3.12±0.46 | <0.05 |
| | $7^{th}$ day | 1.31±0.19 | 2.03±0.35 | <0.05 |
| UCH-L1 (ng/mL) | $4^{th}$ day | 2.85±0.41 | 4.15±0.62 | <0.05 |
| | $7^{th}$ day | 2.13±0.35 | 3.06±0.47 | <0.05 |
| Tf (μg/mL) | $4^{th}$ day | 2.45±0.35 | 3.98±0.62 | <0.05 |
| | $7^{th}$ day | 2.10±0.39 | 3.31±0.38 | <0.05 |
| Ft (ng/mL) | $4^{th}$ day | 203.51±33.94 | 267.51±34.29 | <0.05 |
| | $7^{th}$ day | 174.28±23.15 | 223.48±31.21 | <0.05 |

### 3.2. Neurotrophic index

On the $4^{th}$ day and $7^{th}$ day after the treatment, the context of NTF-$\alpha$, BDNF, NGF and IGF-I in serum of the two groups were determined by Elisa kit. The contents of NTF-$\alpha$, BDNF, NGF and IGF-I in the serum of the intervention group were significantly higher than that in the control group.There were statistically significant differences in serum NTF-$\alpha$, BDNF, NGF and IGF-I in the serum of the two

groups after 4 days and 7 days after treatment(Table 2).

**Table 2**
The comparison of the neurotrophic index in the serum after treatment.

| Index | Time | Intervention group | Control group | *P* |
|---|---|---|---|---|
| NTF- α (ng/mL) | 4th day | 2.91±0.39 | 2.11±0.32 | <0.05 |
| | 7th day | 3.65±0.51 | 2.62±0.36 | <0.05 |
| BDNF (ng/mL) | 4th day | 6.21±0.79 | 4.58±0.57 | <0.05 |
| | 7th day | 8.31±1.02 | 6.96±0.89 | <0.05 |
| NGF(ng/mL) | 4th day | 0.81±0.11 | 0.63±0.09 | <0.05 |
| | 7th day | 1.18±0.16 | 0.84±0.11 | <0.05 |
| IGF-I(nmol/L) | 4th day | 46.49±6.82 | 34.12±4.85 | <0.05 |
| | 7th day | 60.21±7.95 | 47.48±7.92 | <0.05 |

### *3.3. Oxidative stress index*

On 4th day and 7th day after the treatment, The contents of SOD, GPx, CAT, $OH^-$, $O_2^-$, MDA and AOPP in serum of the two groups were measured by radioactive immunoprecipitation kit. The difference was known by *t*-text: The content of SOD, GPx and CAT in the serum of the intervention group were significantly higher than that in the control group, and the contents of $OH^-$, $O_2^-$, MDA and AOPP were significantly lower than those in the control group. The content of SOD, GPx, CAT, $OH^-$, $O_2^-$, MDA and AOPP in serum of the two groups were significant different 4 days and 7 days after treatment.(Table 3)

**Table 3**
The comparison of oxidative stress index in the serum after the treatment.

| Index | Time | Intervention group | Control group | *P* |
|---|---|---|---|---|
| SOD(U/L) | 4th day | 98.31±11.28 | 70.25±8.94 | <0.05 |
| | 7th day | 124.16±15.38 | 89.51±11.28 | <0.05 |
| GPx(U/L) | 4th day | 68.33±9.22 | 46.24±6.48 | <0.05 |
| | 7th day | 83.14±11.41 | 63.51±8.96 | <0.05 |
| CAT(U/L) | 4th day | 41.92±5.65 | 32.39±5.94 | <0.05 |
| | 7th day | 53.19±7.92 | 40.19±6.58 | <0.05 |
| $OH^-$(U/L) | 4th day | 175.82±23.52 | 236.38±33.85 | <0.05 |
| | 7th day | 141.27±19.94 | 204.52±31.29 | <0.05 |
| $O_2^-$(U/L) | 4th day | 70.29±9.93 | 95.18±11.28 | <0.05 |
| | 7th day | 58.58±7.13 | 75.83±9.57 | <0.05 |
| MDA(μmol/L) | 4th day | 12.38±1.85 | 19.82±2.57 | <0.05 |
| | 7th day | 9.38±1.24 | 14.21±1.89 | <0.05 |
| AOPP(μmol/L) | 4th day | 30.29±4.85 | 46.58±6.84 | <0.05 |
| | 7th day | 21.32±3.84 | 31.25±3.95 | <0.05 |

## 4. Discussion

Oxidative stress response is an important secondary pathological factor in acute craniocerebral injury. Anti-oxidative treatment is also considered as an important means to improve the prognosis of patients with acute craniocerebral injury. The occurrence of local oxidative stress response in brain injury is characterized by the continuous mass production of oxygen free radicals which can be directly promoted by local hematoma formation after trauma, red blood cell damage and increased iron ion release; Inflammatory reaction activation and inflammatory cell infiltration can produce oxygen free radicals[6-7]. The compression of the hematoma affects the blood flow and increases the production of oxygen free radicals through the xanthine oxidase[8-9]. Vitamin C and vitamin E are two commonly used vitamins clinically which have antioxidant effect. Vitamin C is a water-soluble antioxidant. On the one hand, it can directly mediate the oxidation-reduction reaction and hydroxylation reaction, thereby eliminating the oxygen free radicals. On the other hand, it can increase the expression of iNOS and increase NO generation, and thus play an anti-oxidation role[10]. Vitamin E is a fat-soluble antioxidant, which can interrupt the oxidation chain reaction of oxygen free radicals to lipid and reduce the oxidative damage of local tissues[11].

In the above study, we used large doses of vitamin C and vitamin E as adjuvant therapy for patients with acute craniocerebral injury. In order to clarify the value of vitamin C and vitamin E in the treatment of acute craniocerebral injury, we first displayed the changes in the degree of nerve injury after treatment by analyzing the index of nerve injury. NSE and S100B are the most widely used nerve injury markers in clinic, respectively located in neurons and glial cells. The former participates in the regulation of cell energy metabolism and the latter participates in the regulation of intracellular calcium homeostasis. The destruction of neurons and glial cells after traumatic brain injury will result in increased release of NSE and S100B[12-14]; NCB is the specific expression of beam albumen inside nerve cell, and It has the function of carrying oxygen and supplying oxygen to neurons[15-16]. The main existing forms of iron ions in erythrocytes are Tf and Ft , brain trauma can cause massive destruction of red blood cells, the breakdown of hemoglobin and the increase of Tf and Ft. rain trauma can cause massive destruction of red blood cells, the breakdown of hemoglobin and the increase of Tf and Ft[17-18]. From the comparison of nerve injury indexes in serum on 4th day and 7th day, we can know: The contents of NSE, S100B, NGB, UCH-L1, Tf and Ft in the serum of the intervention group were significantly lower than those in the control group. This indicates that adjuvant therapy of large doses of vitamin C and vitamin E can reduce the degree of neurological impairment in patients with acute craniocerebral injury.

In the traumatic brain injury, the reconstruction of the nerve function depends on a variety of proliferation effects of nerve cell factor. Cerebral local neurons and glial cells can synthesize and secrete NTF- α , BDNF and NGF plays, IGF-I in and so on a variety of cytokines and mediates a variety of the growth of nerve cells. The damage to neurons and glial cells by craniocerebral injury will decrease the secretion of nerve cell cytokines and affect the reconstruction of nerve function. The function of NTF- α is to improve the nutritional status of nerve cells and enhance the

ability of cells to tolerate damage[19]. The function of BDNF and NGF is to promote the proliferation and regeneration of neurons and the growth of axons, which is beneficial to the repair of nerve function[20-21]. The function of IGF-I is to promote the proliferation of nerve cells, endothelial cells and other cells, which is conducive to the establishment of functional repair and collateral circulation in the process of injury[22]. In order to further clarify vitamin C and vitamin E treatment in acute craniocerebral injury in the course of nerve protective effect, we have compared the content of neurotrophic index in serum in the two groups of patients after treatment for 4 days and 7 days, and according to the results, we can know: The contents of NTF-$\alpha$, BDNF, NGF and IGF-I in the serum of the intervention group were significantly higher than that in the control group. This means that the adjuvant treatment of high doses of vitamin C and vitamin E can reduce the patients with acute craniocerebral injury of nerve tissue damage and destruction, increase neurotrophic the secretion of cytokines, which can improve nerve nutrition state, is advantageous to the neural functional recovery and reconstruction.

Vitamin C and vitamin E have antioxidant effects, which can promote the scavenging of oxygen free radicals and inhibit the oxidation of lipids and proteins. $OH^-$, $O_2^-$ is the main form of oxygen free radicals in the body and craniocerebral local injury generated a lot of $OH^-$, $O_2^-$, which can oxidize with lipid and protein, to produce by-products of MDA, AOPP and cause tissue damage[23-25]. At the same time, a large number of generated oxygen radicals will continue to consume the antioxidant enzyme SOD, GPx and CAT in local tissues[26]. In order to define the vitamin C and vitamin E treatment of acute craniocerebral injury in the course of the reaction of oxidative stress, we compared the context of oxidative stress indicators in serum of the patients from two groups on the $4^{th}$ day and $7^{th}$ day after treatment, and the result showed: The content of SOD, GPx and CAT in the serum of the intervention group was significantly higher than that in the control group, and the contents of $OH^-$, $O_2^-$, MDA and AOPP were significantly lower than those in the control group. This indicates that high-dose vitamin C and vitamin E adjuvant therapy can reduce oxidative stress response in patients with acute craniocerebral injury, reduce the generation of oxygen free radicals and increase the expression of antioxidant enzymes. Large doses of vitamin C and vitamin E are used in the treatment of acute craniocerebral injury, which can reduce the degree of nerve injury and oxidative stress in the course of disease, and improve the neurotrophic state.

## Conflict of interest statement

The authors report no conflict of interest.

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