Original Article
Effects of plasma exchange on serum NO and TGF-β₁ of severe hepatitis patients

Meng Gao*, Hao Tang*, Rong Huang, Rong Gui, Yunfeng Fu

The Third Xiangya Hospital of Central South University, Changsha, Hunan Province, P. R. China. *Equal contributors and co-first authors.

Received December 6, 2016; Accepted December 28, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: Objective: To investigate the mechanism of plasma exchange in the treatment of severe hepatitis, by studying the changes of serum nitric oxide (NO) and transforming growth factor-β₁ (TGF-β₁) in patients with severe hepatitis before and after plasma exchange. Methods: Select 50 cases of normal control group, 35 cases of acute severe hepatitis group, 33 cases of chronic severe hepatitis, 23 cases of early severe hepatitis group, 20 cases of medium severe hepatitis group and 25 cases of late severe hepatitis group; among them, the plasma exchange group and control group were both of 34 cases. The level of serum TGF-β₁ was measured by enzyme-linked immunosorbent assay, serum NO level was detected by Griess. Compare levels of serum NO and TGF-β₁ between the groups, and carry out statistical analysis. Results: The levels of serum NO, TGF-β₁ of patients in each group were higher than that of normal control group (P<0.05), besides, the expressions of serum NO (151.5±44.65 ng/L) and TGF-β₁ (176.7±45.24 ng/L) in late severe hepatitis patients were the highest in different stages of severe hepatitis groups. Levels of serum NO and TGF-β₁ in patients with severe hepatitis treated with plasma exchange were significantly decreased. Compared with those before treatment, the difference was statistically significant (P<0.05). As for the treatment group, the expression levels of serum NO and TGF-β₁ in the patients with effective treatment were significantly lower than that before treatment (P<0.05), and the levels of serum NO and TGF-β₁ in the patients with ineffective treatment were not significantly changed compared with those before treatment. Conclusion: The effects of plasma exchange on the expression of serum NO and TGF-β₁ were effective, besides, it significantly reduced the damage of inflammatory reaction to liver cells and improved the prognosis of patients with severe hepatitis. Hence, the detection of NO and TGF-β₁ indicators is helpful in evaluating the conditions as well as guiding treatment strategies.

Keywords: Severe hepatitis, plasma exchange, nitric oxide (NO), transforming growth factor-β₁

Introduction

Severe hepatitis is one of the most serious complications after hepatitis B virus (HBV) infection, which is characterized by the necrosis or apoptosis of a large number of liver cells, moreover, it can cause acute and chronic liver failure. It has serious conditions, quick development speed, poor prognosis and high mortality rate [1, 2]. It was reported that the pathogenesis of severe hepatitis was complicated which may closely related to the damage of immune system, pathogen invasion, etc. And Cytokine plays a vital role in the development of severe hepatitis. A variety of pathogenic microorganisms and endotoxin can stimulate the release of a large number of cellular factors of mononuclear macrophage, such as serum NO and β₁ (TGF-β₁), further aggravated the damage on liver cells [3, 4]. Other studies have indicated that TGF-β₁ was associated with the pathogenesis of chronic liver disease, and could inhibit the regeneration of liver cells [5, 6]. Therefore, TGF-β₁ may be the key to regulate the regeneration of liver cells in patients with severe hepatitis. NO is a kind of inorganic gas information molecule. It is widely distributed in immune inflammatory cells during the development of severe hepatitis. NO is oxidized in body fluid, forming nitrate, nitrate ion and superoxide anion. The reactive nitrogen intermediate has great cytotoxicity, and plays an important role in the pathogenesis of severe hepatitis. It results in poor efficacy of severe hepatitis after medi-
Effects of plasma exchange on serum NO and TGF-β₁

Table 1. Comparison of general data of patients between the two groups (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin (g/L)</th>
<th>Prothrombin activity (%)</th>
<th>GPT (U/L)</th>
<th>GOT (U/L)</th>
<th>TBil (μmol/L)</th>
<th>Complications (cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment group</td>
<td>29.1±7.3</td>
<td>27.8±7.5</td>
<td>785.2±26.5</td>
<td>585.2±21.8</td>
<td>278.8±75.8</td>
<td>20</td>
</tr>
<tr>
<td>Control group</td>
<td>28.7±7.7</td>
<td>26.6±8.8</td>
<td>773.6±35.2</td>
<td>615.1±27.2</td>
<td>262.5±70.1</td>
<td>18</td>
</tr>
</tbody>
</table>

There was no significantly statistical difference between the two groups in general clinical data such as age, sex, biochemical parameter and complications (ascites, hepatic encephalopathy, hepatorenal syndrome and hemorrhage) and it was comparable, see Table 1. The patients in the treatment group were given plasma exchange who were hospitalized within one week as well as given medical comprehensive treatments like protecting the liver, declining the enzyme, decreasing icterus, maintaining the balance of electrolyte, water and acid-base, replenishing energy and albumin, and anti-infection. The control group was only treated by medical comprehensive treatments after hospitalization. Patients with severe hepatitis were divided into early stage, middle stage and late stage according to their clinical manifestations, which were respectively 23 cases, 20 cases and 25 cases. There was no significantly statistical difference to patients among the different stages in the basic data and it was comparable; all the indexes of liver function were normal in the control group with 50 cases of health checkup patients (Table 1), including 24 cases of male and 26 cases of female, whose age range was from 26.8 to 38.7 years old and mean age was (35.9±3.5) years old.

Inclusion criteria and elimination criteria

Inclusion criteria: Liver failure caused by different kinds of reasons, in accordance with the diagnostic criteria of severe hepatitis; less than 30 s of thrombogenic time; in accordance with indication of plasma exchange and no contraindication; patients and their family members informed consent in this study. Exclusion criteria: Severe obstacles of cirrhotic function; cardiovascular and cerebrovascular diseases; pregnancy or frail elderly patients.

Methods

Measurement of levels of serum NO and TGF-β₁: Patients' levels of serum NO and TGF-β₁ in
Effects of plasma exchange on serum NO and TGF-\(\beta_1\)

Table 2. Comparison of levels of serum NO and TGF-\(\beta_1\) in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>NO (ng/L)</th>
<th>TGF-(\beta_1) (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>50</td>
<td>75.5±18.20</td>
<td>90.74±28.32</td>
</tr>
<tr>
<td>Acute severe hepatitis group</td>
<td>35</td>
<td>87.8±17.65*</td>
<td>123.7±30.52*</td>
</tr>
<tr>
<td>Chronic severe hepatitis group</td>
<td>33</td>
<td>107.9±42.87*</td>
<td>144.5±35.41*</td>
</tr>
</tbody>
</table>

Note: Compared with the control group, *P<0.05; compared with the acute severe hepatitis group, #P<0.05.

Each group were measured before and after plasma exchange. The operation methods were as follows: firstly, 5 ml of fasting venous blood was collected and stored at room temperature. The serum was then isolated by centrifugation at 3000 r/min and the sample was stored at -20°C for inspection. Secondly, the levels of serum TGF-\(\beta_1\) were measured by ELISA (enzyme-linked immunosorbent assay) and the concentration was expressed in ng/L. The human TGF-\(\beta_1\) ELISA kit was purchased from ADL Company (USA) and the operation was strictly in accord with the instructions. Thirdly, the levels of serum NO were measured by Griess and concentration was expressed in ng/L. The NO kit was purchased from the Shenzhen Jingmei Biotech Co. Ltd and the operation was strictly in accord with the instructions.

**Plasma exchange treatment**

WLXGX-888 exchange device, plasma separator and blood return tubes were provided by the Beijing Weili New Century Science & Tech. Deve. Co. Ltd. Patients were performed in the supine position after installing and connecting the exchange tubes correctly. Then the vital signs including blood pressure, heart rate and respiratory function were supervised when patients were undergoing the femoral vein catheterization. 5 mg of dexamethasone was injected for anti-allergic treatment before the plasma exchange and moderate amount (10%) of calcium gluconate would be injected to avoid complications when necessary.

Patients' Y-type double lumen catheters were connected correctly and the dosages of heparin were calculated according to the illness severity, the general dosage in the first time was ranged from 10 mg to 15 mg and then doses of 5 mg were needed at hourly intervals. The plasma bags were connected and then the exchange device was started, the parameters were set as follows: the blood flow rate ranged from 70 ml/min to 100 ml/min; the plasma separation rate ranged from 20 ml/min to 30 ml/min; the plasma exchange volume was 3000 ml/time. The protamine should be used to neutralize the heparin after the plasma exchange, achieving the recovery of patients' blood clotting function. The plasma exchange took 3 hours every time and patients should complete the plasma exchange for three times during the first two weeks.

**Observation indicators**

Patients' levels of serum NO and TGF-\(\beta_1\) were measured before and after the treatment. The effectiveness of treatment was evaluated by the short-term effect of artificial liver support system. And clinical cure and improvement were identified as the effective treatment. Automatic discharge without significant effects or death was identified as the ineffective treatment.

**Statistical methods**

Data were analyzed by using SPSS17.0 statistical software. Measurement data were expressed by mean ± standard deviation (\(\bar{X} \pm S\)); comparison between two groups was analyzed by t test. One-way analysis of variance was applied to compare data among groups; Chi-square test was used for the comparison of rates. P<0.05 indicated statistically significant difference.

**Results**

Comparison of levels of serum NO and TGF-\(\beta_1\) between each group

The levels of serum NO and TGF-\(\beta_1\) in each group were higher than those in the control group, and the differences were statistically significant (P<0.05). Compared with the levels of serum NO and TGF-\(\beta_1\) in the acute severe hepatitis group, those in the chronic severe hepatitis group showed a significant rise, which was statistically significant (P<0.05), as shown in Table 2.
Effects of plasma exchange on serum NO and TGF-β₁

Table 3. Comparison of levels of NO and TGF-β₁ of severe hepatitis patients at different stages (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>NO (ng/L)</th>
<th>TGF-β₁ (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>50</td>
<td>75.5±18.20</td>
<td>92.74±28.32</td>
</tr>
<tr>
<td>Early severe hepatitis group</td>
<td>23</td>
<td>126.7±38.42*</td>
<td>126.41±27.85*</td>
</tr>
<tr>
<td>Medium severe hepatitis group</td>
<td>20</td>
<td>132.4±40.69**</td>
<td>157.5±35.83***</td>
</tr>
<tr>
<td>Late severe hepatitis group</td>
<td>25</td>
<td>151.5±44.65*,#</td>
<td>176.7±45.24*,#</td>
</tr>
</tbody>
</table>

Note: Compared with the control group, *P<0.05; compared with the early severe hepatitis group, **P<0.05; compared with the medium severe hepatitis group, ***P<0.05.

The expression levels of serum NO and TGF-β₁ increased with the progress of the severe hepatitis clinical stage expression, and the serum expression of NO (151.5±44.65 ng/L) and TGF-β₁ (176.7±45.24 ng/L) in the late severe hepatitis group were the highest. See Table 3.

Comparison of levels of serum NO and TGF-β₁ before and after treatment in the two groups of patients

The serum NO level of severe hepatitis patients in treatment group reduced from (137.7±37.7) ng/L before the treatment to (59.5±11.52) ng/L, and the expression of TGF-β₁ also reduced from (158.5) to (111.2±38.82) ng/L. The differences were statistically significant; in addition, compared with that before treatment, the levels of serum NO and TGF-β₁ in the control group reduced after treatment, but the differences were not significant (P>0.05). See Figure 1.

The expressions of serum NO and TGF-β₁ in the treatment group when it was effective or ineffective

In the treatment group, the recent effective rate was 61.8% (21/34) when patients underwent plasma exchange and internal comprehensive treatment. Among them, the serum expressions of NO and TGF-β₁ in the ineffective group were significantly higher than that in the effective group and the differences were statistically significant (P<0.05). Compared before treatment, there was no significant change in the levels of serum NO and TGF-β₁, when the serum expression of NO and TGF-β₁ in the effective group significantly reduced, the differences were statistically significant (P<0.05). See Figure 2.

Discussion

There is high fatality rate in patients with severe hepatitis and the pathogenesis of severe hepatitis is very complex. In addition to the immune damage mechanism, inflammatory cytokines plays a key role in the development of severe inflammation. Casde reaction caused by the
cytokine networks is an important reason that leads to secondary liver injury [11]. Therefore, reducing the expression level of inflammatory factors is conducive to successfully rescue the patients with severe hepatitis. Plasma exchange is an artificial liver treatment therapy in the process of clinical treatments at present. Its' mechanism is to use the powerful regeneration of liver cells and reversible liver damages, through the principle of mechanical purification to help patients recover liver function [12].

NO, as inorganic gas information molecules, has extremely strong fat soluble, can penetrate the cell membrane and play a direct role in the process; when the severe hepatitis occurs and develops, NO which has an induced effect widely distributed in the immune inflammatory cells [13]. In the healthy body, the NO synthase expression level of immune inflammatory cell is extremely low; in the immune inflammation pathological state, endotoxin or immune factors will stimulate the inflammatory cells to produce a lot of NO synthase and mediate the high expression of NO through inflammatory cell. After oxidizing, NO derives acid radical which has stronger cytotoxicity, thereby damaging the liver cells in the incidence of severe hepatitis [14]. Excessive NO will cause great damage to liver cells by inhibiting the mitochondrial respiratory chain, inducing apoptosis of liver cells and producing reactive nitrogen intermediate [15].

TGF-β\(_1\) produced by reactive hepatic stellate cells and hepatic stellate cells is a multifunctional cell growth regulation factor [16]. It was reported that TGF-β\(_1\) played a role in the regulation of liver fibrosis and liver cells necrosis as well as had a close connection with the occurrence of liver cancer [17, 18]. Some animal studies displayed that the expression of TGF-β\(_1\) significantly increased after hepatectomy and had positive correlation with inhibition degree of DNA synthesis [19, 20], demonstrating that TGF-β\(_1\) could inhibit regeneration of liver cells and was bad for the prognosis of severe hepatitis [21, 22]. Some other studies showed that liver cells damage was the promoter factor of TGF-β\(_1\) in hepatic stellate cells or hepatic stellate cells [23, 24]. In the study, compared with the control group, the level of serum NO and TGF-β\(_1\) of patients in each severe hepatitis group had obviously increased with statistical difference (P<0.05). Also, the levels of serum NO and TGF-β\(_1\) in early, medium and late severe hepatitis groups had significant increased, with statistical differences (P<0.05). It was found that the expression of serum NO and TGF-β\(_1\) gradually increased with the staging progress of severe hepatitis and its expression level was the highest in the late severe hepatitis group. Other studies reported that the levels of serum NO and TGF-β\(_1\) significantly increased under the circumstance of severe hepatitis patients' deterioration or death; whereas the levels of serum NO and TGF-β\(_1\) could restore patients to the healthy body under the circumstance of severe hepatitis patients' healing or good prognosis [25, 26]. These results suggested that serum NO and TGF-β\(_1\) could be regarded as biochemical parameters of severe hepatitis patients and their expression levels might had positive correlation with degree of severe hepatitis.
Effects of plasma exchange on serum NO and TGF-β₁

In the study, there were 34 cases in which the level of serum NO and TGF-β₁ of severe hepatitis patients decreased after plasma exchange. Compared to the pre-treatment level, there was significant difference (P<0.05). During the process of plasma exchange for severe hepatitis, we purified the blood by separating plasma and importing fresh plasma so as to help alleviate patient’s condition. However, with the development of disease, the expression of inflammatory cytokines such as serum NO and TGF-β₁ gradually increased. Although plasma exchange could remove the inflammatory cytokines, there were lots of cytokines remained which did harm to liver cells with inflammatory cascade reaction. It might be an important factor to influence the recent effects of severe hepatitis. In the study, the effective rate of plasma exchange for severe hepatitis was 61.8%. In the invalid patients or exacerbation of patients, even if they underwent the plasma exchange, serum NO and TGF-β₁ still produced a lot or could not be effectively removed in the liver because of less residual liver tissue, little improved liver function, resulting in further damage of liver cells.

In conclusion, serum NO and TGF-β₁ could be regarded as biochemical parameters of severe hepatitis patients. Plasma exchange for severe hepatitis could significantly decrease the levels of serum NO and TGF-β₁. We could value the degree, effects and prognosis of severe hepatitis patients by evaluating and analyzing the levels of serum NO and TGF-β₁. In this study, there are still some limitations like the little sample sizes. It needs experiments which are multicenter, have randomized control groups and large sample sizes to further demonstrate. With the further research of the functions of serum NO and TGF-β₁, people can employ more ways to eliminate or inhibit the damage to human organs caused by the excessive expression of serum NO and TGF-β₁ so that the survival rates of the severe hepatitis patients could be further improved.

Acknowledgements

This paper is supported by the Fundamental Research Funds for the Central Universities of Central South University (2016zzts559).

Disclosure of conflict of interest

None.

Address correspondence to: Rong Gui and Yunfeng Fu, The Third Xiangya Hospital of Central South University, No. 138, Tongzipo Road, Yuelu District, Changsha 410013, Hunan Province, P. R. China. Tel: +86-0731-88638888; Fax: +86-0731-88638888; E-mail: guiron_g@126.com (RG); fuyfeng427@163.com (YFF)

References

[11] Nakamoto N. Role of inflammatory macrophages and CCR9/CCL25 chemokine axis in...
Effects of plasma exchange on serum NO and TGF-β1


[23] Baron AV, Osipov NV, Yashchenko SV, Koko-tukha YA, Baron UJ, Puzyr AP, Olkhovskiy IA and Bondar VS. Adsorption of viral particles from the blood plasma of patients with viral hepatitis on nanodiamonds. Dokl Biochem Biophys 2016; 469: 244-246.

