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Original Article

Impacts of early anticoagulant therapy on tissue perfusion in patients with sepsis

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Abstract: This study aims to investigate the impacts and mechanism of early anticoagulant therapy (EAT) on tissue perfusion in patients with sepsis. A total of 224 patients with severe sepsis were randomly divided into the control group (n=105) and the treatment group (n=119). The randomization was performed using computer-generated random numbers. The platelet counting (PLT), D-dimer, blood lactic acid (BLA) and active bleeding of the two groups were comparatively analyzed. The general situations of the two groups were similar. The active bleeding rate of the treatment group was significantly lower than the control group (10.1% *vs.* 4.7%, $P<0.05$), while there was no significant difference in mortality. PLT of the control group showed a progressive decrease at 1 d after treatment ($P<0.05$ or $P<0.01$), while that of the treatment group had no significant change. D-dimer of the control group was continuously maintained at a high level, which was significantly decreased until the 10th d compare with that before treatment ($P<0.05$). D-dimer of the treatment group exhibited significant decreasing on the 3rd d of the treatment ($P<0.05$), and restored to normal on the 10th day. BLA of the control group decreased slowly, and restored to normal until the 10th day, while that of the treatment group was significantly decreased on the 3rd d of the treatment ($P<0.05$), and restored to normal on the 5th day. The application of heparin sodium anticoagulant therapy in the early stage of sepsis could significantly inhibit the reduction of PLT and the increasing of D-dimer and BLA, thus improving the tissue perfusion and reducing the risks of active bleeding.

Keywords: Sepsis, heparin sodium, platelet, D-dimer, tissue perfusion, blood lactic acid

Introduction

The strong systemic inflammatory response is the important feature of patients with sepsis, which in turn activated the coagulation system. These two factors would promote mutually, thus leading to the disseminated intravascular coagulation (DIC) [1], as well as the microcirculation disorder in various organs [2, 3], which would affect the tissue perfusion, and finally result in multiple organ dysfunction syndrome (MODS) and mortality [4, 5]. It could be seen that the microcirculation status in patient with sepsis had become an important symbol towards the prognosis, the earlier the improvement of microcirculation status and tissue perfusion, the higher the successful treatment rate might be achieved [6-9], certain studies used NO, nitroglycerin and other drugs to improve the microcirculation in patients with sepsis, while the effects were not satisfactory [10, 11], this study tried to investigate the impacts and mechanisms of early heparin therapy (EAT) on the tissue reperfusion in patients with sepsis.

Methods

General information

A total of 279 patients with sepsis, admitted into the intensive care unit (ICU) of our hospital from 2010 to 2013, were selected. Exclusion criteria: died within 3 days after the onset of sepsis; with malignant cancer; with severe liver and kidney dysfunction before the onset of sepsis; platelet counting (PLT) >300 × 10^9/L after admitted into ICU; exhibited fresh bleeding wounds in surgery or occurred irritable ulcerative bleeding or other active bleeding before or during treatment. A total of 224 patients were included into the study, including 132 males

Table 1. Comparison of general conditions between the two groups

Items	Control group (n=119)	Treatment group (n=105)
Gender (cases)		
M	74	68
F	45	47
Age ($\bar{x}$±s, years)	53.3±14.5	51.5±12.3
Usage of ventilators [% (cases)]	53.9 (64)	49.4 (52)
CRRT rate (%)	23.5 (28)	25.7 (27)
APACHE II score ($\bar{x}$±s, points)	25.1±5.9	24.3±5.4
Infection site [cases (%)]		
Respiratory tract	57 (47.9)	48 (45.7)
Abdominal cavity	35 (29.4)	32 (30.5)
Urinary tract	14 (11.8)	10 (9.5)
Blood	9 (7.6)	7 (6.7)
Others	18 (15.1)	17 (16.2)
Bacterial species [cases (%)]		
G^+	45 (37.8)	38 (36.2)
G^-	72 (60.5)	62 (59.0)
Fungus	6 (5.0)	7 (6.7)
Mixed	15 (12.6)	14 (13.3)

Note: G^+: Gram-positive bacteria; G^-: Gram-negative bacteria.

Table 2. Bleeding and mortality of the 2 groups (%, cases)

Group	Active bleeding rate	Mortality rate
Control	10.1 (12)	20 (105)
Treatment	4.7 (5)[a]	16 (119)

Note: Compared with the control group at the same period, [a]P<0.05.

and 92 females; aged 13 to 75 years (with the mean age 51.4±19.9 years). The patients were randomly divided into the control group (n=105) and the treatment group (n=119) using computer-generated random numbers. During treatment, 18 patients in the control group died, and 16 patients in the treatment group died, therefore excluded; the control group exhibited 21 cases of liver dysfunction, and the treatment group exhibited 19 cases of liver dysfunction, thus excluded during blood lactic acid (BLA) analysis. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Sun Yat-sen University. Written informed consent was obtained from all participants.

Diagnostic criteria

The diagnostic criteria of sepsis and septic shock referred to the standards defined by the International Conference of Sepsis in 2001 [12]. Criteria of active bleeding: intracranial bleeding; gastrointestinal or urinary tract bleeding, hemoglobin was decreased by 10 g/L or more; respiratory tract bleeding, resulting in the decreasing of oxygenation index (PaO_2/FiO_2) and significant shortness of breath.

Treatment

All patients were given the treatments of antibiotics, fluid resuscitation (expansion), vasoactive drugs, nutritional support and organ support. The treatment group was intravenously infused heparin 3 $mg/kg^{-1}\cdot d^{-1}$ on D1, and continued for 10 days; the control group was given the same amount of saline. The patients with renal failure and oliguria in the 2 groups received the continuous renal replacement therapy (CRRT), and *in vitro* anticoagulation during CRRT to minimize the impacts of CRRT. *In vitro* anticoagulation: 50 mg heparin was added into 50 ml of 5% glucose for the continuous intravenous infusion twice a day; the ratio of heparin and protamine was 1:0.9. For patients with renal failure requiring CRRT in treatment group, besides early application of heparin sodium, the in vitro anticoagulant therapy was performed for CRRT itself. 50 mg heparin dissolved in 50 ml of 5% glucose was used for continuous intravenous infusion, twice a day; the ratio of heparin and protamine was 1:0.9.

Detection indicators

PLT, D-dimer, BLA and active bleeding of the 2 groups were detected before treatment, and on D1, 3, 5 and 10 after treatment; the acute physiology and chronic health evaluation II (APACHEII) were used for the scoring.

Statistical analysis

The measurement data were expressed as mean ± standard deviation (SD), the intragroup comparison used the group t test, the intergroup comparison used paired t test, the counting data were compared with the Chi-square test, with P<0.05 considered as the statistically significant difference.

Table 3. Comparison of PLT changes during treatment (/L × 10^9) ($\bar{x}$±s)

Group	Before treatment	D1	D3	D5	D10
Control	134±38	124±28	81±16[c]	65±17[d]	53±17[d]
Treatment	137±40	125±25	131±32[a]	132±35[a]	141±39[b]

Note: Compared with the control group at the same period, [a]*P*<0.05, [b]*P*<0.01; compared with the same group before treatment, [c]*P*<0.05, [d]*P*<0.01.

Table 4. Comparison of D-dimer Change (μg/L) ($\bar{x}$±s)

Group	Before treatment	D1	D3	D5	D10
Control	810.7±129.8	835.3±134.8	751.3±102.8	671.0±88.4	577.2±72.6[c]
Treatment	823.7±131.2	792.1±106.6	531.5±71.8[a,c]	347.8±54.9[b,d]	241.2±34.3[b,d]

Note: Compared with the control group at the same period, [a]*P*<0.05, [b]*P*<0.01; compared with the same group before treatment, [c]*P*<0.05, [d]*P*<0.01.

Table 5. Comparison of BLA changes (mmol/L) ($\bar{x}$±s)

Group	Before treatment	D1	D3	D5	D10
Control	4.0±1.6	4.1±1.8	3.6±1.4	3.3±1.3	2.1±0.8[d]
Treatment	3.9±1.6	3.8±1.5	2.7±1.1[a,c]	2.2±0.8[b,d]	1.7±0.6[b,d]

Note: Compared with the control group at the same period, [a]*P*<0.05, [b]*P*<0.01; compared with the same group before treatment, [c]*P*<0.05, [d]*P*<0.01.

Results

General conditions

The basic information (such as age, gender), disease (such as infection site, bacterial species and APACHEII scores, etc.) and treatment methods (except for heparin) between the 2 groups showed no statistically significant difference (*P*>0.05), thus these 2 groups were comparable (**Table 1**).

Bleeding and mortality

As shown in **Table 2**, the active bleeding rate in the treatment group was significantly lower than that in the control group (*P*<0.05). Although the mortality rate in the treatment group was lower than the control group, there was no statistical significance.

PLT

The treatment of the control group was prolonged, PLT showed a progressive decrease (*P*<0.05 or *P*<0.01). PLT of the treatment group decreased on D1 of therapy, began to increase on D3, and returned to the pre-treatment level on D10. The PLT values of the treatment group on D3, 5 and 10 were significantly higher than that of the control group (*P*<0.05 or *P*<0.01, **Table 3**).

D-dimer

D-dimer of the control group was continued to be maintained at a high level, and significantly decreased than that before treatment until D10 (*P*<0.05). D-dimer of the treatment group exhibited significant decrease compared with that before treatment on D3 (*P*<0.05), and returned to normal on D10, while the values of the treatment group on D3, 5 and 10 were significantly lower than the control group (*P*<0.05 or *P*<0.01, **Table 4**).

BLA

BLA of the control group decreased slowly, and returned to normal until D10, while exhibited significant difference from that before treatment (*P*<0.01). BLA of the treatment group was significantly decreased on D3 compare to that before treatment (*P*<0.05), and restored to normal on D5, the values of the treatment group on D3, 5 and 10 exhibited significant difference from those of the control group at the same period (*P*<0.05 or *P*<0.01, **Table 5**).

Discussion

It was found in clinical practice that there was certain incidence of overt DIC in patients with sepsis, which was further increased in patients

with severe sepsis, and the incidence was the highest in patients with septic shock. The coagulation disorders in patients with sepsis was caused by the bacterial endotoxins, which stimulated the monocytes/macrophages to release large amounts of inflammatory mediators, thus causing the systemic inflammation, and the inflammatory mediators would then cause the coagulation disorders in patients with sepsis, and finally led to the formation of DIC. The coagulation disorders and the inflammatory reactions might promote and influence mutually, thus forming the pathophysiological process of sepsis together, and ultimately leading to MODS and death [13].

The nonfatal changes in sepsis, also namely the rapid thrombosis, would lead to the reduction of PLT. The patients with severe conditions would exhibit the increasing of vascular permeability, therefore, the patients would already die of cardiavascular collapse before the occurrence of DIC. Most patients would appear as progressive, continuous reduction of PLT and other coagulation disorders (such as the increasing of D-dimer) [14]. Many factors could lead to the PLT reduction in patients with sepsis, such as infection and inflammation-caused bone marrow suppression and hemophagocytosis, the application of intravascular catheters and drugs were also the reasons, and in sepsis, the extensive vascular thrombosis was the common cause towards the reduction of PLT in medical and surgical ICU [15-17].

This study found that the application of heparin in the early stage of sepsis could decrease PLT on D1, which would returned to normal on D3 gradually; while the control group continued to decrease, and the PLT value of the treatment group on D3 was significantly higher than the control group, which were more significant on D5 and 10. The application of heparin in the early stage of sepsis could decrease D-dimer rapidly on D3, which was significantly increased on the day of onset, and the D-dimer value could decrease down to normal on D10; while the control group could maintain it at a higher level for a long time, and still maintained significantly higher than the treatment group on D3, 5 and 10, indicating that the application of heparin in early stage of sepsis could improve patients' blood clotting functions, prevent the formation of micro-thrombosis, and significantly inhibit the reduction of PLT and the increase of D-dimer, thus ultimately significantly inhibiting the active bleeding rate of the treatment group than the control group. So, it could be considered that the early application of heparin might inhibit the formation of micro-thrombosis, block the occurrence of implicit DIC or progressive DIC, and reduce the incidence of active bleeding.

Experiments showed that the animals with sepsis existed poor tissue perfusion in the early stage, and it was the key factor for the early organ dysfunction, the OPS (orthogonal polari zation spectral) imaging technique could intuitively reveal the significant reduction of small blood vessels that were continuously perfused [18]. The severe microcirculation disorder would lead to tissue hypoxia, thus the aerobic metabolism was reduced and the anaerobic glycolysis was increased, resulting in the increased production of lactic acid.

This study found that BLA of the treatment group was decreased rapidly, and returned to normal on D5. While BLA of the control group was decreased slowly, which was still significantly higher than normal on D5. In order to exclude the impacts of liver dysfunction on BLA, the patients with liver dysfunction had been removed during the study, the results suggested that the early application of heparin could significantly inhibit the increasing of BLA.

Mechanism of heparin in impacting the tissue perfusion in patients with sepsis: the patients with severe sepsis would occur severe hemodynamic changes (i.e. septic shock), as well as serious micro-thrombosis (i.e. DIC), the above two factors could cause serious shortage of tissue perfusion, and lead to tissue hypoxia and increasing anaerobic metabolism, thus increasing the production of lactic acid. In addition, severe shock and DIC would damage the functions of liver, kidney and other organs, thus resulting in further obstacles to remove BLA, and ultimately leading to significant increasing of BLA. This study found that the early application of heparin might improve the functions of blood clotting in patients with sepsis, reduce the formation of micro-thrombosis, inhibit the reduction of PLT and the increase of D-dimer, improve the tissue perfusion and reduce the generation of BLA. This might be one of the

mechanisms through which heparin could affect the tissue perfusion in patients with sepsis.

This study found that such anti-clotting therapy as heparin could improve patients' coagulation and tissue perfusion, reduce the active bleeding. Currently, some scholars advocated that the application of heparin towards DIC plus severe sepsis should be the earlier the better, and the small dose would be better, except for the serious bleeding, heparin should not be suspended and should be maintained until DIC was completely controlled [18]. However, the impacts of EAT on the prognosis of patients with sepsis were still uncertain, Wiedermann and Kaneider [19] confirmed, through random controlled clinical trials, that the antithrombin anticoagulant therapy might increase the survival rates of DIC patients with severe sepsis and patients with septic shock. Most studies had found that the anticoagulant therapy could not improve the mortality. Zhang and Ma [20] found that the early application of low molecular weight heparin could improve the coagulation functions in patients with severe sepsis, but could not improve the survival rate. De Pont and Schuttz [21] treated severe sepsis patients with heparin, misoprostol, or the combination of these two, and found that the endothelial cell functions could be improved, while the survival rate was not improved. This study also found that EAT could not reduce the mortality. Therefore, it still needed further study to investigate the application of heparin towards the patients with sepsis.

Taken together, this study indicated that the early application of heparin in patients with sepsis could improve patients' coagulation and tissue perfusion, furthermore improve organ function and reduce active bleeding rate, but could not improve the survival rate.

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Disclosure of conflict of interest

None.

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