

Effects of Alprostadil combined with Edaravone on inflammation, oxidative stress and Pulmonary function in patients with traumatic hemorrhagic shock

Dong Luo¹, Qingmei Pan¹, Linlin Wang¹, Wenfeng Zhao¹, Wentao Bao^{2*}

¹ Intensive Care Unit, Feicheng Hospital Affiliated to Shandong First Medical University, Feicheng, 271600, China

² Respiratory Intensive Care Unit, Tai'an City Central Hospital, Tai'an, 271000, China

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ABSTRACT

The study aimed to explore the roles of alprostadil combined with edaravone in inflammation, oxidative stress and Pulmonary function in patients with traumatic hemorrhagic shock (HS). 80 patients with traumatic HS treated in to Feicheng Hospital Affiliated to Shandong First Medical University and Tai'an City Central Hospital from January 2018 to January 2022 were enrolled and divided into observation group (n=40) and control group (n=40) according to the randomized control method. Patients in the control group were given alprostadil alone (5 g alprostadil + 10 mL normal saline) on the basis of conventional treatment, while those in the observation group received edaravone (30 mg edaravone + 250 mL normal saline) in addition to treatment in the control group. The patients in both groups were treated via intravenous infusion once a day for 5 days. 24 hours (h) after resuscitation, venous blood were collected to detect serum biochemical indicators such as blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Enzyme-linked immunosorbent assay (ELISA) was conducted to determine serum inflammatory factors. Lung lavage fluid was collected to examine Pulmonary function indicators such as myeloperoxidase (MPO) and matrix metalloproteinase-9 (MMP-9) activity and to observe theoxyxygenation index (OI). Blood pressure was measured at admission and 24 h after surgery. The observation group had significantly lowered serum BUN, AST and ALT ($p < 0.05$), the content of serum interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) as well as oxidative stress indexes like superoxide dismutase (SOD) and malondialdehyde (MDA) ($p < 0.05$) and Pulmonary function indicators ($p < 0.05$) but overtly increased content of SOD and OI. Furthermore, the blood pressure in the observation group dropped to 30 mmHg at admission and rose to the normal range. Alprostadil combined with edaravone effectively reduces inflammatory factors and improves oxidative stress and pulmonary function in patients with traumatic HS, whose efficacy is significantly better than that of alprostadil alone.

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Introduction

Damage and ischemia/reperfusion of massive tissues will lead to post-traumatic hemorrhagic shock (HS), and uncompensated hemorrhage after trauma makes the innate immune system to initiate an excessive systemic inflammatory response syndrome (1), acute and severe hypovolemia and anemia give rise to insufficient perfusion and oxygenation of vital organs (2). Tissue hypoperfusion and hypoxia due to the imbalance between systemic oxygen supply and oxygen consumption may lead to irreversible cell damage and the release of pathological active substances, including cytokines, reactive oxygen species and nitric oxide (3, 4), which induces multiple organ dysfunction syndromes (MODS) when progressing to a severe stage. MODS is one of the leading causes of death in patients in intensive care units (5). The mechanisms of post-traumatic HS in triggering SIRS and MODS have been extensively studied, but have not been fully clarified yet. Overactivation of neutrophils and endothelial cells leads to up-regulation of cell surface adhesion molecules, including selectins and integrins, which promote the adhesion of neutrophils to the inflammatory vascular endothelium

and thus result in the extravasation of neutrophils to important organs, followed by degranulation and release of proteases and other hydrolases, as well as reactive oxygen species, and synthesis of inflammatory cytokines, which are key steps of shock in mediating the development and progression of MODS (6). Inflammatory reactions caused by post-traumatic HS may result in pulmonary dysfunction (7), further promoting the progression of HS.

Severe trauma-induced HS, one of the most common causes of death (8), is characterized by unstable hemodynamics, cellular hypoxia and weakened organ function. Trauma and HS can stimulate endogenous inflammatory cytokines such as IL-1 β and tumor necrosis factor- α (TNF- α) released by the system (9). Previous small animal studies have found the presence of pulmonary inflammation after HS and subsequently investigated the effects of anti-inflammatory interventions (10). Related studies in primates describing the pathophysiology and hemodynamic variables in detail are rare and reveal conflicting results (11, 12). In the case of HS, the production of free radicals leads to an increase in oxidative stress that is related to high mortality and morbidity rates of HS, the whose prognosis of which can be improved through the

* Corresponding author. Email: baowentao2022@yandex.com

treatment with anti-oxidative stress drugs (13). For patients with severe HS, it is critical to restore central hemodynamics via liquid displacement and inhibition of inflammatory cascade activation. Edaravone, a free radical scavenger, has been successfully applied in the treatment of acute cerebral ischemia (14). Much evidence has manifested that edaravone exerts a protective effect even after administration after HS ischemia caused by severe trauma (15). A study has discovered that edaravone is capable of suppressing the lipid peroxidation chain reaction in endothelial cells, neurons, and glial plasma membranes (16) and has an antioxidant effect, which can decrease levels of cellular oxidative stress (17). Studies have proved that alprostadil, i.e. prostaglandin E1 (PGE1), has a variety of biological functions and a good preventive and therapeutic effect on liver, pulmonary and kidney fibrosis. Its protective effect on the lungs is mainly to repress neutrophils and inflammatory cytokines produced. However, the effects of alprostadil combined with edaravone on inflammation, oxidative stress and pulmonary function in patients with traumatic HS are rarely studied, and the specific mechanisms of action remain unclear.

In this study, biochemical markers, serum inflammatory factors, oxidative stress indexes and pulmonary function indicators were detected in enrolled patients with traumatic HS so as to prove that alprostadil combined with edaravone can effectively reduce inflammatory factors and improve oxidative stress and pulmonary function in patients with traumatic HS, with evidently better efficacy than that of alprostadil alone, providing the theoretical basis and experimental evidence for the treatment of traumatic HS.

Materials and Methods

Clinical data

This clinical research protocol was approved by the Ethics Committee of the Tai'an City Central Hospital. The study subjects were 80 patients with traumatic HS admitted to Feicheng Hospital Affiliated with Shandong First Medical University and Tai'an City Central Hospital from January 2018 to January 2022. These patients were enrolled after signing the informed consent and divided into an observation group (n=40) and a control group (n=40) in accordance with the randomized controlled method. Inclusion criteria: Shock patients with a heart rate ≥ 105 beats/min and a systolic blood pressure =70-90 mmHg before admission and an emergency infusion volume <1,000 mL in the Emergency Department. Exclusion criteria: Patients younger than 15 years of age, pregnant women or those receiving an intravenous infusion of isotonic crystalloid solution >2,000 mL, any colloid or blood product on the scene before treatment with the study solution, undergoing prehospital cardiopulmonary resuscitation, with severe hypothermia (body core temperature <28°C), drowning, asphyxia due to hanging, with burns >20% of the total body surface area, or unable to receive intravenous injection. All clinical specimens in this study were collected according to the Declaration of Helsinki after getting approval from patients and their families. The specific clinical data of patients (Table 1) collected at admission included age, gender, weight and injury mechanism.

Therapeutic methods

Conventional first aid scheme: The airway was kept open for a high concentration of oxygen inhalation for sufficient oxygen inhalation. Blood volume was quickly replenished to restore effective circulation via the instillation of isotonic saline at a high rate (1,000 mL was infused in 45 min). While replenishing blood volume, hemostasis was carried out as soon as possible. The bleeding was temporarily stopped with a tourniquet for initial correction of the shock, followed by radical hemostasis. The patients in the control group were treated with alprostadil alone (5 g alprostadil + 10 mL normal saline) via intravenous infusion once a day for 5 d on the basis of conventional first aid. Those in the observation group underwent edaravone treatment (30 mg edaravone + 250 mL normal saline) via intravenous infusion once a day for successive 5 d in addition to the treatment in the control group.

Detection of serum blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Serum biochemical indicators, including liver function indexes, are affected by many diseases, and traumatic HS is not an exception. After awakening the patients venous blood (5 mL) was collected from the arms in Eppendorf (Ep) tubes containing anticoagulant and centrifuged at room temperature and 2,000 g for 15 min, and the supernatant was collected for the detection of liver function indicators such as BUN, AST, and ALT, providing important theoretical references for early treatment.

Determination of serum inflammatory factors via enzyme-linked immunosorbent assay (ELISA)

After the patient arrives at the hospital, collect venous blood from the arm (5 mL) into an EP tube containing anticoagulant, followed by centrifugation at room temperature and 2,000 g for 15 min. Next, the supernatant was collected to detect serum inflammatory factor indexes interleukin-6 (IL-6), IL-1 and TNF- α according to the instructions of the ELISA kit (purchased from the Nanjing Jiancheng Bioengineering Institute). Finally, the absorbance of each group was measured using a microplate reader.

Examination of serum oxidative stress indexes through ELISA

Venous arm blood (5 mL) was collected in EP tubes containing anticoagulant after the patients regained consciousness and centrifuged at room temperature and 2,000 g for 15 min. After that, the supernatant was collected to determine the change in the content of serum oxidative stress indexes malondialdehyde (MDA) and superoxide

Table 1. Clinical data.

Parameter	Control group	Observation group
Number of samples	40	40
Number of male patients	30	25
Average age	45 \pm 15	44 \pm 18
Average weight (Kg)	55 \pm 11.5	54 \pm 12.5
Injury type		
Blunt injury	22	18
Sharp injury	18	22

dismutase (SOD) in accordance with the instructions of the ELISA kit (bought from the Nanjing Jiancheng Bioengineering Institute). Lastly, the absorbance in each group was measured using the microplate reader.

Detection of myeloperoxidase (MPO) and matrix metalloproteinase-9 (MMP-9) activity in pulmonary lavage fluid by ELISA

Monocyclic surgery (Olympus) was conducted using a fiber bronchoscope. Under the guidance of the eyepiece, 37°C normal saline (50 mL) was injected into the left lower lobe, and 15 s later, it was immediately withdrawn. The last 5 mL of the lavage fluid was collected and centrifuged at 1,000 g and low temperature for 10 min, and the supernatant was collected. Subsequently, the activity of MPO and MMP-9 in the lungs was determined according to the instructions of the ELISA kit purchased from Nanjing Jiancheng Bioengineering Institute. Next, the absorbance in each group was read using an automated microplate luminometer (EL340, BIO-TEK Instruments). The values obtained were analyzed according to the manufacturer's instructions.

Examination of oxygenation index (OI)

All patients were subjected to endotracheal intubation in the intensive care unit to receive intermittent synchronized intermittent mandatory ventilation (SIMV), and improvements in the clinical symptoms of the patients were observed. During the mechanical ventilation, attention was paid to vibration expectoration. At 24 h after revival, the arterial OI of patients in each group was observed and recorded. Subsequently, spontaneous respiration was allowed.

Measurement of blood pressure at admission and 24 h after regaining consciousness

The cuff was wrapped around the right upper arm of the patient and the rubber ball was squeezed to drive air into the cuff to gradually raise the mercury column on the blood pressure gauge until there was no pulsation of the radial artery on the stethoscope. Then, the air in the cuff was slowly released, and the value of the mercury column on the blood pressure meter recorded at this time was the systolic pressure. The slow release of the air was continued, and the value on the blood pressure meter recorded when the sound suddenly weakened was the diastolic pres-

sure.

Statistical analysis

All raw laboratory data recorded were processed by SPSS 22.0 analysis software and subjected to multiple comparisons. The laboratory results obtained were expressed as mean \pm standard deviation ($\bar{x} \pm SD$), and $p < 0.05$ indicated that the difference was statistically significant. Graphpad Prism 8.0 was employed for histograms.

Results

Serum BUN, AST and ALT detected

The results (Table 2) showed that the content of ALT, AST and BUN was significantly lowered in the observation group ($p < 0.05$).

Serum inflammatory factors determined via ELISA

As shown in Table 3, the levels of IL-1, IL-6 and TNF- α were evidently lower in the observation group than those in the control group ($p < 0.05$), while they were still higher in the control group than normal reference values.

Serum oxidative stress indicators examined by ELISA

The results of SOD and MDA (Figure 1) revealed that in the observation group, the MDA content was notably decreased ($p < 0.05$), while the expression of SOD was

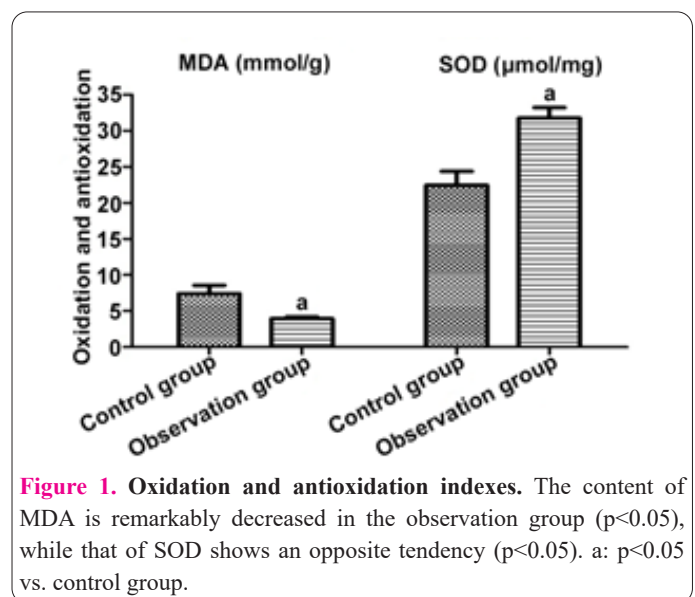


Figure 1. Oxidation and antioxidant indexes. The content of MDA is remarkably decreased in the observation group ($p < 0.05$), while that of SOD shows an opposite tendency ($p < 0.05$). a: $p < 0.05$ vs. control group.

Table 2. Changes in serum ALT, AST and BUN.

Group	AST (U/L)	ALT (U/L)	BUN (mmol/L)
Control group	101 \pm 1.2	99 \pm 1.5	28.12 \pm 4.58
Observation group	70 \pm 2.3 ^a	68 \pm 1.8 ^a	12.64 \pm 1.72 ^a

Note: The observation group has overtly declined the content of ALT, AST and BUN ($p < 0.05$). a: $p < 0.05$ vs. control group.

Table 3. Levels of serum TNF- α , IL-1 and IL-6.

Group	IL-1 (mg/L)	TNF- α (fmol/mL)	IL-6 (mg/L)
Control group	39.5 \pm 5.6	35.5 \pm 5.4	45.4 \pm 1.4
Observation group	25.5 \pm 3.8 ^a	28.7 \pm 6.4 ^a	32.4 \pm 1.0 ^a

Note: The observation group exhibits clearly reduced levels of IL-1, IL-6 and TNF- α ($p < 0.05$). a: $p < 0.05$ vs. control group.

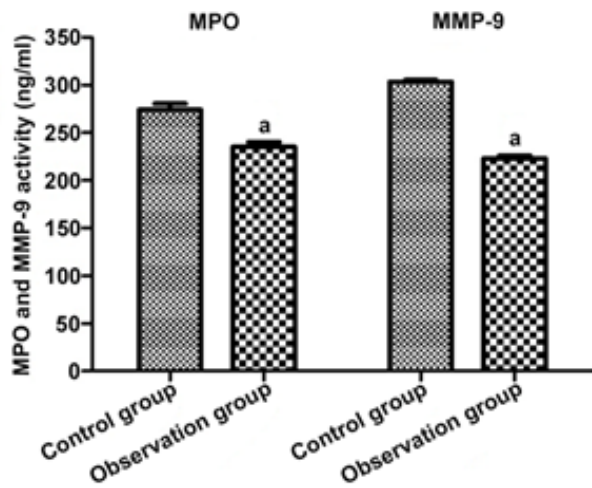


Figure 2. MPO and MMP-9 activity. The content of MPO and MMP-9 is distinctly lowered in the observation group ($p<0.05$). a: $p<0.05$ vs. control group.

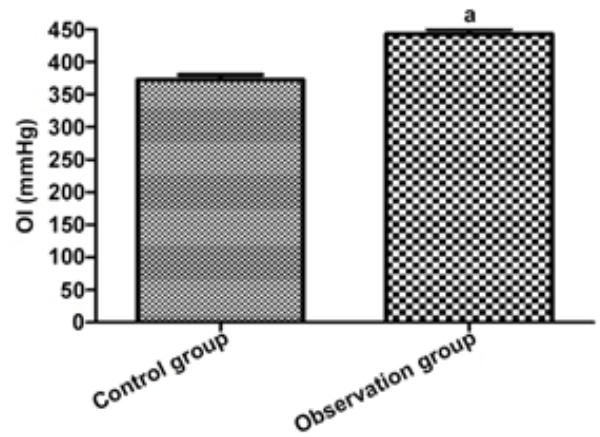


Figure 3. OI. In the control group, the OI is elevated to about 380 mmHg which is basically close to the normal reference value (400 mmHg), and in the observation group, the OI is increased to about 420 mmHg, which is overtly higher than that in the control group ($p<0.05$). a: $p<0.05$ vs. control group.

clearly elevated ($p<0.05$).

MPO and MMP-9 activity determined through ELISA

The results of MPO and MMP-9 are shown in Figure 2. The content of MPO and MMP-9 was markedly reduced in the observation group ($p<0.05$).

Arterial OI detected

The OI was reduced to 300 mmHg at admission in the observation group. The OI Roseto was about 380 mmHg in the control group, which was close to the normal reference value of 400 mmHg, and it was raised to about 420 mmHg in the observation group, which was significantly higher than that in the control group ($p<0.05$) (Figure 3).

Blood pressure measured at admission and 24 h after coming round

Based on Table 4, the blood pressure decreased to 30 mmHg at admission, while at 24 h after coming round, the systolic and diastolic pressures were remarkably higher in the observation group than those in the control group ($p<0.05$).

Discussion

Traumatic hemorrhage leads to impaired tissue perfusion and oxygen transmission, thus resulting in cell hypoxia (18). In such a case, oxidative stress and inflammatory responses participate in the progression of MODS after fluid resuscitation and tissue ischemia/reperfusion (19). The lungs are very susceptible to the harmful effects of hypovolemic shock. Quantitative studies of pulmonary injury in HS rats have shown that the recruitment of pro-inflammatory cytokines and activated neutrophils

into lung tissues have a positive relation to the severity of hemorrhage. Besides, similar pathophysiological changes are detected in the lungs even after fluid resuscitation for HS. Excessive inflammatory factors, including neutrophils, IL-6 and tissue necrosis factor and oxidative stress, are found in mouse models. TNF- α is indispensable for the progression of inflammation in HS rats, and IL-6 triggers the excessive production of other inflammatory mediators (20). It was found in this study that the levels of ALT, AST and BUN were evidently reduced in the observation group, suggesting that the application of alprostadil combined with edaravone in the treatment of traumatic shock is able to overtly improve liver function indicators. In addition, the results of this study showed that the content of IL-1, IL-6 and TNF- α was notably decreased in the observation group, which was almost close to that in the control group, implying that alprostadil combined with edaravone applied in the treatment of traumatic shock is capable of reducing the inflammatory cells secreted by inflammatory cytokines due to shock, avoiding the irreversible damage to cells caused by their excessive production and stimulating various anti-inflammatory substances to resist damage caused by inflammation. Moreover, the patient's blood pressure had dropped to 30 mmHg at admission, and the systolic and diastolic pressures were clearly elevated after treatment with alprostadil combined with edaravone. Furthermore, the OI had been reduced to 300 mmHg at admission, and it was raised to about 380 mmHg in the control group (which was close to the normal reference value 400 mmHg) and about 420 mmHg in the observation group after treatment with alprostadil combined with edaravone, which was significantly higher than that in the control group. The above results indicate that after the

Table 4. Blood pressure of patients (mmHg).

Group	Systolic pressure	Diastolic pressure
At admission	50 \pm 1.5	30 \pm 0.5
Control group	80 \pm 1.0	55 \pm 0.5
Observation group	92 \pm 0.5 ^a	63 \pm 1.0 ^a

Note: At admission, the blood pressure decreases to 30 mmHg, while at 24 h after coming round, the systolic and diastolic pressures are markedly higher in the observation group than those in the control group ($p<0.05$). a: $p<0.05$ vs. control group.

emergency treatment of patients, the blood pressure and OI have basically returned to the normal state and will not be life-threatening, and more treatment can be carried out subsequently. In the early stage after hemorrhage, the decrease in OI during bleeding can be interpreted as the change in blood distribution in the lungs and the mismatch between ventilation and perfusion. A study has shown that the OI ratio exhibits an obvious decrease at 3-5 h after HS, resulting in acute pulmonary injury (21), which is in line with the findings of this study.

HS and subsequent ischemia/reperfusion injury are correlated with oxidative stress in organs (22). It has been suggested that such oxidative stress is the basic mechanism of organ damage in HS. Oxygen radicals are involved in HS and affect subsequent resuscitation. SOD is extensively expressed, and MDA is able to counteract the effects of SOD and is cytotoxic (23). Wu et al (24) demonstrated that HS and resuscitation induce significant oxidation in the lungs of rats, while Panteli et al (25) reported that the oxidation state of the kidneys is uninfluenced after HS. In antioxidant case of HS, the treatment can ameliorate organ oxidative stress and achieve better efficacy. Antioxidant therapy with permeable membrane-free radical scavengers improves the prognosis of HS in rats (26). In this study, evident oxidative stress was found in the lungs during HS and resuscitation, which was alleviated after the application of alprostadil combined with edaravone. Moreover, the MDA content distinctly declined, while the level of SOD increased significantly in the observation group, with oxidative stress significantly improved.

MPO is mainly distributed in neutrophils, whose cytoplasmic granules contain massive MPO. Therefore, the increased content of MPO in lung lavage fluid suggests the increase in neutrophil content, and its excessive accumulation will lead to inflammation, so it can serve as a predictor of lung inflammatory damage (27). MMP plays an important role in the degradation of extracellular matrix and the destruction of proteolytic enzymes that are stimulated by pro-inflammatory cytokines. Fully activated MMP may be conducive to lung injury. Moreover, certain components (such as IL-1, TNF and lipopolysaccharide) can specifically induce and trigger the up-regulation of MMP-3 and MMP-9, which is an important factor for irreversible pulmonary injury in HS (28). In this study, the content of MPO and MMP-9 content was markedly reduced in the observation group, demonstrating that alprostadil combined with edaravone relieves inflammatory cell infiltration, inhibits oxidative stress, and improves HS-induced pulmonary injury in patients with HS. Although this study confirmed the effects of alprostadil combined with edaravone on inflammation, oxidative stress and pulmonary function in patients with traumatic HS, there are still some shortcomings. Subsequently, in vivo laboratory, animal experiments are needed to verify such effects. Specifically, such effects should be further investigated from multiple levels and multiple perspectives via molecular experiments such as immunofluorescence, flow cytometry, and EMSA, providing important theoretical and experimental bases for subsequent related studies.

In conclusion, alprostadil combined with edaravone attenuates inflammatory cell infiltration, represses oxidative stress and mitigates pulmonary injury in HS patients, providing a theoretical basis for the prevention and treatment of HS and new thought for subsequent further research.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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