

Original Article

Changes of endothelial glycocalyx and effect of fluid resuscitation combined with norepinephrine on endothelial glycocalyx in early septic shock

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Abstract: Objective: To investigate effect of fluid resuscitation on glycocalyx of rabbits with early septic shock. Methods: Twenty-four rabbits were randomly divided into control group, sepsis group (LPS group), sepsis +10 ml treatment group (10 ml group), and sepsis +30 ml treatment group (30 ml group). Effects of norepinephrine combining with resuscitation fluid on atrial natriuretic peptide (ANP) and plasma syndecan-1 were investigated. Results: ANP level was the lowest in control group, followed by LPS group, 10 ml group and 30 ml group. Plasma syndecan-1 concentration was the lowest in control group, followed by 10 ml group, 30 ml group and LPS group. The expression of syndecan-1 in renal tissues was decreased in the order of control group, 10 ml group, 30 ml group and LPS group. The expression of syndecan-1 and angiotensin-1 in renal tissues was decreased in the order of control group, 10 ml, 30 ml and LPS group. The expression of angiotensin-2, matrix metalloproteinase-2, 7 and intercellular adhesion molecule-1 was increased in 10 ml group, followed by 30 ml group and LPS group. Univariate analysis demonstrated significant differences in ANP among the four groups (all $p < 0.05$), and between-group differences were found (all $P < 0.05$). Conclusion: Vascular endothelial glycocalyx was shedding in rabbits with septic shock. Timely resuscitation with crystalloids in combination with norepinephrine exerts a protective effect on endothelial cell and glycocalyx. An adequate dose of fluid is recommended, as opposed to excessive fluid that is detrimental. Excessive infusion of fluid aggravated glycocalyx damage, further increasing tissue permeability and leading to poor prognosis.

Keywords: Fluid resuscitation, endothelial glycocalyx, norepinephrine, prognosis, septic shock

Introduction

Severe sepsis and septic shock are vital clinical issues in critical care settings. Successful management of patients with severe sepsis or septic shock in intensive care units is based on the following three aspects: timely and reasonable application of antibiotics, hemodynamic support, and infection control. Fluid resuscitation is a hemodynamically key intervention in the early stage of sepsis, however, there has been no consensus concerning the optimal kind of fluid, including the right amount. While rapid optimization of intravascular volume has been shown to improve the prognosis, it is reported that fluid overload often occurs following an early Goal Directed Therapy (EGDT) in the management of severe sepsis and septic shock, which results in poor clinical prognosis [1]. Therefore, it is critical to administer timely and

appropriate fluid resuscitation in terms of sepsis treatment. In this study, the sepsis model was established by intravenous injection of lipopolysaccharide via the marginal ear vein of rabbits. Rabbits were resuscitated with different doses of fluids combined with norepinephrine.

The luminal surface of the endothelial cells (ECs) that line vasculature is lined by a layer of membrane-bound glycoproteins and proteoglycans (glycocalyx). Glycocalyx, a vascular barrier, plays a central role in limiting access of large molecules to EC, preventing leukocyte and platelet adhesion, and limiting tissue edema. However, it is extremely vulnerable to physiological and pathophysiological changes, such as impaired blood flow in major blood vessels [2], enzyme degradation [2-4], activation of matrix metalloproteinases (MMPs) [5], declined levels

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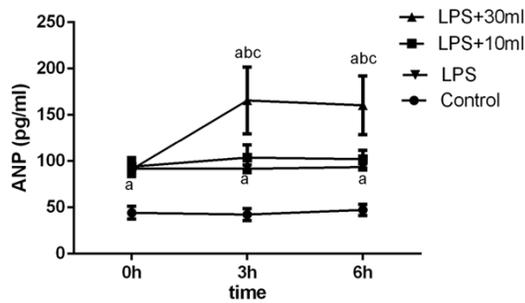


Figure 1. ELISA results of ANP at three time points in the four groups. After comprehensive analysis by two-way ANOVA, there were significant differences among the three groups, at different time points and in interaction between grouping and time points (all $P < 0.05$). The mean value of ANP in each group was increased in a time-dependent manner, which was lower at 0 h than at 3 h and 6 h (all $P < 0.05$), and no significant differences were found at 3 h and 6 h ($p = 0.909 > 0.05$). Note: a, compared with Control, $P < 0.05$; b, compared with 10 ml, $P < 0.05$; c, compared with 30 ml, $P < 0.05$.

of plasma albumin, blood volume overload, which precede glycocalyx degradation, causing a series of inflammatory reactions [6, 7]. In the meantime, it has been found that oxidative stress during sepsis can disrupt glycocalyx [8], leading to increased EC permeability, thereby giving rise to fluid and protein leakage as well as resulting tissue edema.

The ideal resuscitation fluid for sepsis should have capacity to restore intravascular volume and reduce incidence of edema, theoretically repairing the glycocalyx to restore endothelial layer integrity [9]. However, aggressive fluid resuscitation increases the filling pressure of the heart, inducing an elevated atrial natriuretic peptide (ANP) release. Bruegger found that ANP initiated glycocalyx shedding in animal experiments, leading to elevated vascular leakage and permeability [10]. ANP cleaves the membrane-bound proteoglycan and glycoprotein (polyglycoside-1 and hyaluronic acid) on the endothelial glycocalyx, resulting in glycocalyx shedding, which significantly increased the EC permeability. Several previous studies have found the role of leukocytes and endothelium in the inflammatory and ischemia-reperfusion injuries and the association between glycocalyx shedding and the activation of extracellular enzymes, in which MMPs play an essential role [11]. Nonetheless, little is known as for whether different doses of resuscitation fluid in the early septic shock affect EC glycocalyx, giving

rise to different clinical outcomes. The possible mechanisms of different doses of resuscitation fluid on renal EC injury during sepsis remain incompletely understood. To this end, the present study is the first attempt to observe maintenance of tissue perfusion by administering different doses of resuscitation fluid combined with intravenous norepinephrine in rabbits with early septic shock. The expression changes of plasma markers ANP and syndecan-1 were detected, to analyze whether different doses of resuscitation fluid produce different therapeutic effects by means of affecting EC damage. The expressions of syndecan-1, angpt1, angpt2, intercellular cell adhesion molecule-1 (ICAM-1), MMP-2, and MMP-7 in renal tissue were detected by both immunohistochemistry and western blot.

We hypothesized that glycocalyx injury coincided with correlated protein activation on endothelial cells in fluid resuscitation. To verify this hypothesis, effects of different doses of resuscitation fluid on sepsis shock were explored. The possible mechanism of EC glycocalyx shedding during sepsis was investigated, thereby providing an important theoretical basis for options of different doses of resuscitation fluid in early sepsis in clinical practice.

Materials and methods

Animals and grouping

This study has been approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. The experiment was performed in accordance with international ethics standards for animal experimentation. Twenty-four New Zealand white male rabbits (weighing 2.5~3.0 kg) provided by Animal Experimental Center of Hebei Medical University were randomly divided into control group ($n = 6$), sepsis group (LPS group; $n = 6$), sepsis +10 ml treatment group (LPS+10 ml group, hereinafter referred to as 10 ml group; $n = 6$), and sepsis +30 ml treatment group (LPS+30 ml group, hereinafter referred to as 30 ml group; $n = 6$).

Animal model establishment and specimen collection

Rabbits in LPS group and the treatment groups were injected with lipopolysaccharide (3 mg/kg, lipopolysaccharide L-4130, Sigma, USA) via a marginal ear vein to prepare septic shock

Table 1. ELISA results of ANP at three time points in the three groups

Group	0 h	3 h	6 h	Total
Control	44.33 ± 6.66	42.4 ± 6.02	47.28 ± 5.96	44.67 ± 6.2
LPS	92.00 ± 8.32 ^a	91.78 ± 4.00 ^a	93.68 ± 3.24 ^a	92.39 ± 5.38
LPS+10 ml	94.11 ± 9.29 ^a	103.88 ± 13.1 ^a	102.21 ± 9.08 ^a	100.07 ± 10.92
LPS+30 ml	91.24 ± 3.8 ^a	165.64 ± 34.4 ^{a,b,c}	160.48 ± 30.19 ^{a,b,c}	139.12 ± 42.88

Note: a, compared with Control, P<0.05; b, compared with LPS, P<0.05; c, compared with LPS+10 ml, P<0.05.

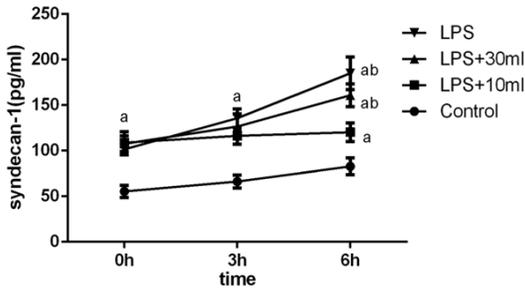


Figure 2. ELISA results of syndecan-1 at three time points in four groups. After comprehensive analysis by two-way ANOVA, significant differences were noted between the two groups, at different time points and in interaction between grouping and time points (all P<0.05). The mean value of syndecan-1 in each group was increased in a time-dependent manner, which was the lowest at 0 h (all P<0.05), followed by at 3 h (all p<0.05) and at 6 h (P<0.05). Note: a, compared with Control, P<0.05; b, compared with 10 ml, P<0.05.

model. 3 ml saline was injected intravenously into the marginal ear vein of rabbits in the control group. When the rabbits shrunk with decreased activity, a loss of appetite and energy, and aggravated dyspnea, urethane anesthesia (4 mg/kg) was administered immediately, followed by timely left carotid artery catheterization, right internal jugular vein catheterization and tracheotomy. After the carotid artery intubation, the model was successfully constructed when the average arterial pressure was monitored <65 mmHg and lactic acid > 3 mmol/L by carotid blood gas analysis. Subsequently, the rabbits in the treatment groups were administered immediately with normal saline at 10 ml/kg/h or 30 ml/kg/h for 1 h, and the average arterial pressure was kept > 75 mmHg. If the blood pressure did not achieve the MAP target, the norepinephrine was initiated. There was no significant difference of norepinephrine usage between two groups. After 1 h, both groups were given 5 ml/kg/h saline. By contrast, the LPS group and the

control group were given only 5 ml/kg/h saline to maintain the fluid circuit. 1 ml of blood samples were collected at 0 h, 3 h and 6 h after model establishment. After centrifugation, supernatant

was collected and placed in a -80°C refrigerator for ELISA assay. Animals were sacrificed at 6 h after the injection of lipopolysaccharide. The renal cortex was harvested and stored in an -80°C refrigerator and 4% paraformaldehyde solution respectively for protein blotting and immunohistochemistry.

Detection of plasma ANP and syndecan-1 by ELISA, syndecan-1 protein in the renal cortex by immunohistochemistry and syndecan-1, angpt1, angpt2, ICAM-1, MMP-2 and MMP-7 in renal tissues by Western blotting

The concentration of plasma ANP at 0 h, 3 h and 6 h and plasma marker syndecan-1 was determined by ELISA assay.

The sample was sliced to a thickness of 4 µm, followed by conventional dewaxing hydration, and exposure to antigen using high-temperature antigen retrieval method. Rabbit anti-rabbit syndecan-1 (1:100 dilution) and biotinylated goat anti-mouse IgG (1:100 dilution) were used as primary and secondary antibody respectively. PBS, as opposed to primary antibody, was used as a negative control. DAB solution was utilized for coloration and the sample was observed under light microscopy. The results showed that positive signal of syndecan-1 was brown particles located in the cytoplasm and cell membrane.

Total protein was extracted by RIPA lysate. The protein concentration was determined by BCA method (1:1000, A1235, ABclonal USA). An equal amount of protein for each group was separated by SDS-PAGE, and then electrotransferred to PVDF membrane, which was incubated with 5% skim milk at 37°C for 2 h. The concentrations were as follows: syndecan-1 antibody (1:1000, A1235, ABclonal, USA), angpt1 antibody (1:1000, AF5184, Affinity, USA), angpt2 antibody (1:1000, A0698, Affinity,

Table 2. ELISA results of syndecan-1 at three time points in four groups

Group	0 h	3 h	6 h	Total
Control	55.47 ± 6.28	66.26 ± 6.77	82.89 ± 8.68	68.21 ± 13.49
LPS+10 ml	108.93 ± 11.22 ^a	116.29 ± 8.8 ^a	120.26 ± 9.77 ^a	115.16 ± 10.54
LPS+30 ml	107.62 ± 8.12 ^a	126.64 ± 13.1 ^a	160.87 ± 12.01 ^{a,b}	131.71 ± 25.03
LPS	101.54 ± 5.75 ^a	135.70 ± 9.77 ^a	185.04 ± 17.13 ^{a,b}	140.76 ± 36.98

Note: a, compare with Control, P<0.05; b, compare with LPS+10 ml, P<0.05.

USA), ICAM-1 antibody (1:1000, ARH4246, AR, USA), MMP-2 (1:1000, ARE6040, AR, USA), and MMP7 antibody (1:1000, A0695, ABclonal, USA). After rinse, TBST was incubated with HPR-labeled IgG secondary antibody (1:3000) at 37°C for 2 h, and ECL chemiluminescence imaging was performed. After the film was scanned, electrophoresis bands were quantitatively analyzed by ImageJ2X software. The ratio of integrated optical density value of the target band to integrated optical density value of β-actin band was used to represent the relative expression of the target protein.

Statistical analysis

SPSS19.0 software was used for statistical analysis. Measurement data were presented as mean ± standard deviation. Two-way ANOVA was adopted to analyze the changes of ANP and syndecan-1 in plasma at different time points. Between-group comparison was performed using one-way analysis of variance and Dunnett t test. Paired t test was used to compare the differences of indicators for each group before and after resuscitation. P<0.05 was considered statistically significant.

Results

Levels of plasma syndecan-1 and ANP were reduced in 10 ml group after fluid resuscitation

ELISA results showed the lowest concentration of ANP in plasma of the control group, followed by the LPS group, whereas the concentration was remarkably lower in 10 ml group than in 30 ml group after resuscitation. Compared with control group, Two-way ANOVA revealed that ANP was lower at 0 h than at 3 h and 6 h in LPS, 10 ml and 30 ml groups (all P<0.05) (Figure 1 and Table 1). Compared with the control group, Plasma syndecan-1 level was significantly increased in LPS group, which was notably up-regulated with the course of the disease.

Fluid resuscitation reduced plasma syndecan-1 level, which was more pronounced in 10 ml group. Statistically significant differences were noted between two groups (P<0.05), at different time points

and in interaction between grouping and time points, as indicated by two-way ANOVA (P<0.05) (Figure 2 and Table 2).

Syndecan-1 expression in rabbit kidney tissue was significantly up-regulated in 10 ml group after fluid resuscitation

Immunohistochemical results confirmed positive signals of syndecan-1 as brown granules predominantly localized in the cytoplasm and/or membrane of renal VEC and glomerular cells. Compared with the normal control group, the expression of syndecan-1 in the renal tissue of LPS group was significantly decreased, which, however, was up-regulated to be higher in 10 ml group than in 30 ml group. (Figure 3).

Fluid resuscitation influenced expression of syndecan-1 and angpt1, angpt2, ICAM-1, MMP-2 and MMP-7 in renal tissues

Western blot indicated that the expression of syndecan-1 and angpt1 protein was significantly lower in LPS group than in normal control group, whereas the expression of angpt2, ICAM-1, MMP-2 and MMP-7 was notably increased, as shown by one-way ANOVA. Fluid resuscitation contributed to significantly increased expression of syndecan-1 and angpt1 protein as well as significantly decreased expression of angpt2, ICAM-1, MMP-2 and MMP-7, in particular, in 10 ml group (P<0.001). (Figures 4 and 5 and Tables 3 and 4).

Discussion

Recently, some studies suggested that some new therapeutic methods aiming to protect the glycocalyx against injury represented a promising direction in clinical medicine. Further, the common interventions in the acutely ill, such as fluids, blood products, and organ-supporting techniques, should be re-evaluated during the course in terms of their EG “friendliness” [12].

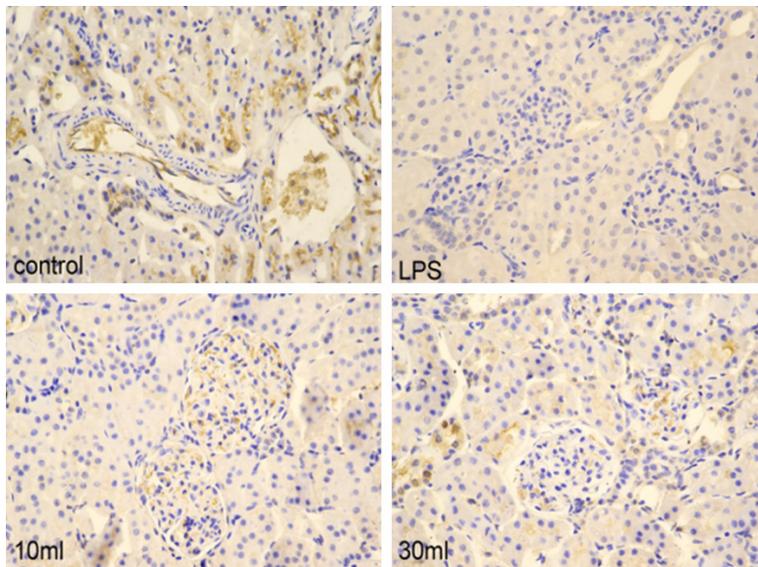


Figure 3. Immunohistochemical results of syndecan-1 in the four groups. The expression of syndecan-1 in the renal tissue was significantly lower in LPS group than in the normal control group. The expression of syndecan-1 protein was up-regulated, which was higher in 10 ml group than in 30 ml group.

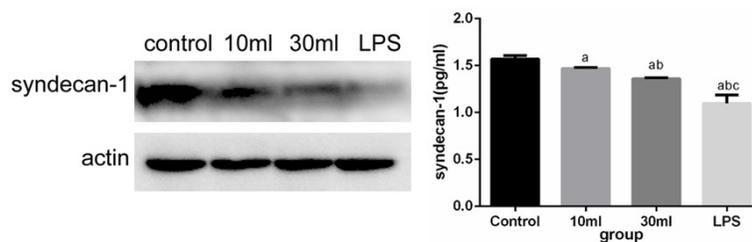


Figure 4. Western blot results in the four groups. The expression of syndecan-1 in renal tissues was decreased in the order of control group, 10 ml group, 30 ml group and LPS group (all $P < 0.001$, between either two groups). The treatment of fluid resuscitation led to significantly increased expression of syndecan-1 particularly in 10 ml group. Note: a, compared with Control, $P < 0.05$; b, compared with 10 ml, $P < 0.05$; c, compared with 30 ml, $P < 0.05$.

To our knowledge, this is the first report on different doses of resuscitation fluid combined with norepinephrine in the early septic shock affect glycocalyx. The purpose of fluid resuscitation is to increase cardiac output and tissue perfusion, providing sufficient oxygen for tissue and cells to maintain organ function. Therefore, fluid resuscitation is the cornerstone of saving life in hypovolemic shock [13]. Inadequate or excessive fluid is likely to cause tissue hypoperfusion. Fluid management also has a “cut-off point”. It’s unlucky that the point is still unclear. Excessive fluid leads to the dilution of the blood, which reduces its ability to carry oxygen and increases the capillary leakage and tissue edema, thus causing more obvious tissue

hypoxia. The above condition is particularly pronounced in the fluid management of septic shock. Early goal-directed therapy (EGDT) [14] improves the survival rate of patients with septic shock, which is challenged by mounting evidence in clinical practice that EGDT may pose a risk of fluid overload for septic patients, thereby increasing medical intervention and hospital mortality [1]. Therefore, conservative fluid strategies based on physiological hemodynamics are sensible, which may reduce patient mortality and improve survival [15, 16].

The glycocalyx on the surface of the vascular endothelium plays a key role in maintaining the intravascular volume and the homeostasis. Several triggers are known to deteriorate the glycocalyx such as fluid overload, ischemia, and TRALI [17]. A serious infection will trigger a series of cascade reactions such as activation of MMPs. MMPs activation and glycocalyx reduction have been reported in LPS-induced sepsis animals and inhibition of its activity can protect the glycocalyx [18]. This suggests that under pathological conditions, up-regulation of MMPs

activity can alter components of glycocalyx, resulting in glycocalyx shedding. In the inflammatory response, the expression of MMPs is active, which leads to degradation of the extracellular matrix and destruction of intercellular junctions [19, 20], thereby causing abnormal leakage, edema and multiple organ failure [21, 22]. Gronski also found that MMP-2, MMP-7 and MMP-9 could directly cleave chondroitin sulfate [23]. Studies by Herbert H. Lipowsky suggested that lower doses of doxycycline inhibited MMPS activity, thus suppressing leukocyte adhesion and glycocalyx shedding [5]. Microcirculatory dysfunction is crucial in severe infection, so it is a hot issue in this field as for how to stabilize the intact structure and func-

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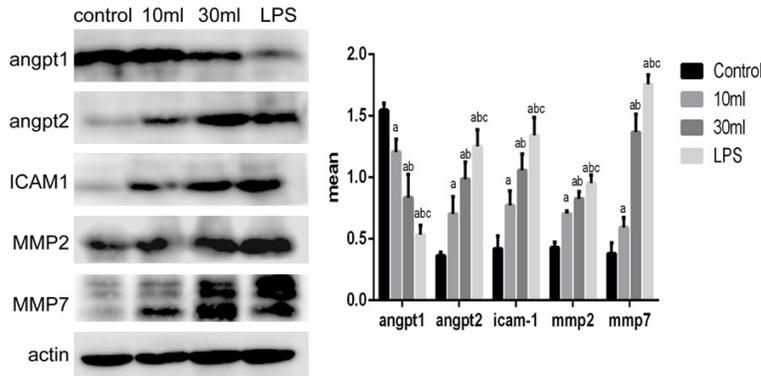


Figure 5. Western blot results in the four groups. The expression of angpt1 protein was significantly reduced, whereas the expression of angpt2, ICAM-1, MMP-2 and MMP-7 protein was remarkably increased in the order of control group, 10 ml group, 30 ml group and LPS group (all $P < 0.05$, between either two groups). The treatment of fluid resuscitation led to significantly increased expression of angpt1 as well as remarkably decreased expression of angpt2, ICAM-1, MMP-2 and MMP-7 protein, particularly in 10 ml group. Note: a, compared with Control, $P < 0.05$; b, compared with 10 ml, $P < 0.05$; c, compared with 30 ml, $P < 0.05$.

tion of EC and reduce the excessive activation and disruption of ECs [24]. The aim of this study was to investigate the effects of different doses of resuscitation fluid combined with norepinephrine on vascular endothelial glycocalyx in rabbits with early septic shock. The results revealed significantly increased plasma syndecan-1 in septic rabbits and remarkably decreased expression of syndecan-1 in renal tissue, suggesting that sepsis was accompanied by vascular glycocalyx shedding that led to increased expression in the plasma. The serum level was up-regulated with the progression of the disease, indicating the significant role of glycocalyx shedding in the development of sepsis. The results indicated remarkably elevated expression of MMP-2 and 7 in the renal tissue of sepsis group and decreased expression of syndecan-1 protein, demonstrating that the glycocalyx shedding was closely related to MMPs overexpression or activity in the course of sepsis. To our knowledge, there have been few studies on the impact of different resuscitation fluid on glycocalyx by activating related proteins, such as MMPs. In the present study, we observed the effect of different doses of resuscitation fluid on EC function and marker protein expression. The results revealed the protective benefit of fluid resuscitation to glycocalyx of EC. Nevertheless, compared with 10 ml group, expression of syndecan-1 in the renal vascular endothelium was decreased, whereas plasma

level of syndecan-1 was higher in 30 ml group. However, the expression of MMP-2 and 7 was significantly different in renal tissues. This suggested that different fluid resuscitation exerted diverse effects on vascular endothelium. Moreover, studies have suggested that glycocalyx can be stabilized by maintenance of normal MMPs during inflammation [25].

ICAM-1, also known as CD54, is a member of the immunoglobulin superfamily and an important adhesion molecule that mediates the adhesion reaction. ICAM-1 is expressed at low levels in resting VEC and exerts its specific biological activity by binding to specific receptors on the surface of VEC. As a transmembrane protein of leukocytes and EC, ICAM1 plays an important role in stabilizing intercellular interactions and promoting the migration of leukocytes and ECs. The results of this study showed that compared with the control group, the expression of ICAM-1 in renal tissue of LPS group was significantly increased, whereas the expression of syndecan-1 protein was reduced, suggesting that glycocalyx shedding might associate with elevated ICAM-1 expression in the course of sepsis. In addition, fluid resuscitation was also found to protect the vascular endothelium. ICAM-1 expression in renal VEC was decreased, whereas syndecan-1 expression was elevated in 10 ml group as compared with 30 ml group, suggesting different roles of various doses of resuscitation fluid in the vascular endothelium.

Angpt1 plays a substantial role in preserving vascular integrity and limiting permeability, whereas angpt2 is the promoter of endothelial activation [26, 27]. In critically ill patients, the release of angpt2 induce breakdown of the vascular endothelial barrier function [28, 29], and is thought to be a marker of sepsis [30]. Furthermore, the increase in angpt2 concentration in plasma is associated with fluid overload, which is responsible for liver dysfunction, coagulation disorders, and acute renal injury. angpt2 activation may enhance vascular leakage, lead-

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Table 3. The expression of syndecan-1 in renal tissues

Tissue	Control	LPS+10 ml	LPS+30 ml	LPS	F	P
Kidney	1.57 ± 0.04	1.47 ± 0.01 ^a	1.36 ± 0.01 ^{a,b}	1.1 ± 0.09 ^{a,b,c}	51.796	<0.001

Note: a, compare with Control, P<0.05; b, compare with LPS+10 ml, P<0.05; c, compare with LPS+30 ml, P<0.05.

Table 4. The expression of angpt1, angpt2, icam1, mmp2 and mmp7 in renal tissues

Tissue	Indicators	Control	LPS+10 ml	LPS+30 ml	LPS	F	P
Kidney	angpt1	1.55 ± 0.06	1.21 ± 0.1	0.84 ± 0.18	0.53 ± 0.07	44.246	<0.001
	angpt2	0.36 ± 0.03	0.7 ± 0.14	0.99 ± 0.14	1.25 ± 0.13	30.908	<0.001
	icam1	0.42 ± 0.1	0.77 ± 0.11	1.06 ± 0.14	1.34 ± 0.14	29.215	<0.001
	mmp2	0.43 ± 0.04	0.7 ± 0.02	0.82 ± 0.06	0.95 ± 0.06	62.430	<0.001
	mmp7	0.38 ± 0.09	0.59 ± 0.08	1.37 ± 0.15	1.75 ± 0.08	116.273	<0.001

ing to more fluid requirements, organ dysfunction and death [31]. John S. et al. have reported that plasma levels of angpt2 were significantly increased in children with septic shock as compared with healthy children, and that angpt2 levels was correlated with prognosis and the severity of the disease. Compared with SIRS or sepsis, plasma levels of angpt2 were remarkably elevated, whereas those of angpt1 were notably lower in children with septic shock [32]. In this study, plasma levels of syndecan-1 in septic rabbits and the expression of angpt2 in the renal tissue were increased, as accompanied with significantly decreased angpt1 expression, indicating glycocalyx shedding during sepsis and an increase in vascular permeability. In the meantime, the expression of angpt2 was lower in 10 ml group than in 30 ml group, whereas the expression of angpt1 was increased, revealing different effects of various doses of resuscitation fluid on the permeability during sepsis.

Bruegger et al. [33] found that in terms of patients undergoing on- or off-pump coronary artery bypass surgery, three major components were shed from the glycocalyx significantly. Based on changes in their concentration and ANP concentration, ANP designated as a mediator initiated this inflammatory release process [33], but its specific mechanism remains poorly understood. Moreover, a number of studies have shown that hypervolemia brought about the release of ANP, inducing short-term glycocalyx degradation by basement MMPs [7]. Daniel Chappell [6] found that hypervolemia promoted the release of ANP, bringing about enhanced glycocalyx shedding, which impaired the vascular

barrier. Therefore, volume loading might not be as safe as assumed and should be rigorously evaluated. It has been found that high doses of resuscitation fluid account for elevated cardiac filling pressure, inducing the release of natriuretic peptide [34, 35], which cleaves the endothelial glycocalyx (predominantly polyglycoside-1 and hyaluronic acid) [10, 33, 36]. The findings of this study on rabbits with septic shock showed the lowest concentration of plasma ANP in the control group after fluid resuscitation, and the notably higher concentration in 30 ml group than in 10 ml group. This suggests that excessive fluid resuscitation increases ANP, which may bring about a series of cascade reactions.

In conclusion, in the present study we noticed glycocalyx shedding of vascular endothelium in rabbits with septic shock, which may be attributed to the up-regulation of MMPs. Resuscitation with crystalloids in combination with norepinephrine exerts protective effects on glycocalyx, which improves endothelial function and reduces vascular permeability. However, adequate fluid is preferable, as opposed to excessive fluid which is detrimental. Excessive fluid infusion aggravated glycocalyx damage, possibly causing iatrogenic injury. The limitations of this study include a relatively small sample size and a lack of a dynamic observation of the glycocalyx after fluid resuscitation for septic shock. Besides, the pathology and physiology of LPS-induced septic shock model might not be totally the same with the septic shock in clinical practice. Therefore, further investigation is needed to guide the clinical practice.

Disclosure of conflict of interest

None.

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