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Effective Combination Therapy of Polymyxin-B Direct Hemoperfusion and Recombinant Thrombomodulin for Septic Shock Accompanied by Disseminated Intravascular Coagulation: A Historical Controlled Trial

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Abstract: Disseminated intravascular coagulation (DIC) and multiple organ failure often occur via the crosstalk between inflammation and coagulation, which is mediated by High Mobility Group Box 1 (HMGB1). In septic shock, Polymyxin-B direct hemoperfusion (PMX-DHP) ameliorates hemodynamics by endogenous cannabinoid adsorption and improves pulmonary oxygenation by indirect cytokine reduction through the adsorption of activated mononuclear cells. However, PMX-DHP has no direct effect on HMGB1 circulating in the plasma. In cases with DIC, recombinant thrombomodulin (rTM), an effective drug for DIC, exerts not only anticoagulation but also antiinflammatory properties via direct anti-HMGB1 activity. Therefore, a combination of PMX-DHP and rTM is expected to block the vicious cycle of a cytokine storm ending up with multiple organ failure in DIC. The aim of this study was to investigate the efficacy of combination therapy for septic shock associated with DIC. This study comprised 22 consecutive patients with sepsis-induced DIC who received PMX-DHP. The initial eight patients were treated without rTM (historical control group), and the following 14 patients were given rTM (rTM group). The baseline Sequential Organ Failure Assessment (SOFA) score or age was not different between both groups. Sixty-day survival rate in the rTM group was significantly higher than that in the control group (85.7% vs. 37.5%, $P = 0.015$). A combination of PMX-DHP and rTM may be effective in septic shock accompanied by DIC and is expected to improve survival rates. **Key Words:** Disseminated intravascular coagulation, High Mobility Group Box 1, Polymyxin-B direct hemoperfusion, Recombinant thrombomodulin, Septic shock.

Sepsis, defined as systemic inflammatory response syndrome (SIRS) incurred by bacteria or their components (1), often leads to severe shock and multiple organ failure. The toll-like receptor (TLR) families on monocytes/macrophages are involved in the beginning of a chain reaction by infection. TLR2 and TLR4 are related with the recognition of Gram-positive and Gram-negative bacteria, respectively. Once infection is recognized irrespective of the kind of infection, the same intracellular activation pathway induces an inflammatory reaction (2).

In sepsis, mononuclear cells/macrophages which are activated via TLR release early mediators such as interleukin-6 (IL-6) and IL-8, which further stimulate mononuclear cells/macrophages. The cells release a late mediator, High Mobility Group Box 1 (HMGB1) (3). Once endothelial cells are injured, endothelial cells also release excessive HMGB1 into the bloodstream.

Wang et al. reported that HMGB1 is not detected in healthy subjects but is recognized at high concentrations in septic patients and at even higher concentrations in fatal septic patients (4). HMGB1 is increased in disseminated intravascular coagulation

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(DIC) cases. Serum concentrations of HMGB1 show a positive correlation with the DIC score or the severity of DIC. In cases with organ failure, the concentration is much higher and seems to be regarded as a prognostic factor (5).

Polymyxin-B direct hemoperfusion (PMX-DHP) has widely been used in Japan as the treatment for septic shock. Recently, its effectiveness has also been reported from other countries (6,7). PMX-DHP is designed to remove endotoxin through direct adsorption. It not only improves hemodynamics by direct removal of endogenous cannabinoid adsorption (8,9), but also reduces early mediators by direct adsorption of activated mononuclear cells (10).

Recombinant thrombomodulin (rTM) (Asahi-Kasei Pharma, Tokyo, Japan), an effective DIC drug, shows anti-HMGB1 activity through accelerating the degradation of HMGB1 by thrombin (11) and inhibiting the binding of HMGB1 with Receptor for Advanced Glycation Endproducts (RAGE) and TLR2/4 (12). We hypothesized that the combination of PMX-DHP and rTM would improve the clinical outcome of septic shock associated with DIC. The purpose of this study was to examine the efficacy of the combination therapy.

PATIENTS AND METHODS

Subjects

We performed a historical control study in a single center. Patients with sepsis-induced DIC who received PMX-DHP in the ICU of Osaka National Hospital, Osaka, Japan, from April 2009 to November 2012 were included in this study. The inclusion criteria were: (i) a known or suspected infection on the basis of clinical data at study entry; (ii) two or more signs of systemic inflammation with at least the presence of sepsis-induced organ dysfunction; and (iii) hematologic dysfunction. All patients fulfilled the criteria of the Japanese Association for Acute Medicine DIC scoring system (Table 1) (13). Patients with untreated HIV infection, severe cytomegalovirus infection, or severe acute pancreatitis were excluded from the analysis ($N = 3$).

From August 2011, 14 patients who met the inclusion criteria were eligible for treatment with rTM (rTM group). Eight other patients who met the same inclusion criteria and who were treated from April 2009 to July 2011 were nominated as the comparison controls (control group). This study followed the principles of the Declaration of Helsinki. This study was conducted with the approval of the institutional review board at Osaka National Hospital, and the board waived the need for informed consent for a retrospective study such as this study.

Interventions

All patients were principally treated according to the strategy of the Surviving Sepsis Campaign Guidelines 2008. In the control group, all patients with septic DIC received synthetic protease inhibitors (sPI) or heparin products with or without antithrombin (AT). In the rTM group, all patients received rTM (0.06 mg/kg per day) with or without sPI and/or AT.

Because we prescribed rTM with the goal of DIC restoration, the administration period was different in each case. Continuous hemodiafiltration (CHDF) was performed as renal replacement therapy in some cases.

Data collection

We defined the day when PMX-DHP was initiated as day 0. Patients were followed up for 60 days after PMX-DHP was initiated. The variables considered to assess the comparability among two groups were age, sex, Sequential Organ Failure Assessment (SOFA) score at the initiation of PMX-DHP, site of infection, and PaO_2/FiO_2 ratio (P/F ratio). We evaluated 60-day survival rates. SOFA scores were recorded each day on day 0 and day 2.

PMX-DHP

Direct hemoperfusion (DHP) by PMX-fiber (Toray, Tokyo, Japan) was carried out at a flow rate of 80 mL/min through the venovenous catheter. Nafamostat mesilate (Sanwakagaku, Aichi, Japan) was used as the anticoagulant in all the cases.

TABLE 1. *Criteria of Japanese Association for Acute Medicine disseminated intravascular coagulation (DIC) scoring system*

	SIRS score	Platelet counts ($\times 10^3/\mu L$)	PT ratio	FDP (μg/mL)
0 point	0–2	≥120	<1.2	<10
1 point	≥3	≥80 but <120 or decreased by more than 30% within 24 h	≥1.2	≥10 but <25
2 point	–		–	–
3 point	–	<80 or decreased by more than 50% within 24 h	–	≥25

Diagnosis of DIC ≥4 points.

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TABLE 2. *Baseline characteristics and therapeutic interventions in the study population*

	rTM group ($N = 14$)	Control group ($N = 8$)	P-value
Age (years)	72.0 (67.8–76.8)	70.0 (65.5–76.5)	n.s.
Male sex	10	6	n.s.
SOFA score	12.0 (10.0–13.3)	12.0 (9.25–17.0)	n.s.
P/F ratio	182.0 (111.8–277.0)	244.6 (160.4–288.8)	n.s.
Site of infection			n.s.
Lung	6	1	
Abdomen	6	7	
Urinary tract	1	0	
Skin and Soft tissue	1	0	
Others	0	0	
Culture			
Gram-positive	9	6	n.s.
Gram-negative	9	7	n.s.
PMX duration (h)	29.5 (15.1–55.0)	7.8 (2.8–20.8)	0.014
Therapeutic interventions			
CHDF	2	5	n.s.
AT	9	4	n.s.

Data are expressed as group median (interquartile range). AT, antithrombin; CHDF, continuous hemodiafiltration; P/F ratio, PaO_2/FiO_2 ratio; rTM, recombinant thrombomodulin; SOFA, Sequential Organ Failure Assessment.

Statistical Analysis

Continuous variables are expressed as group median with interquartile range, except for survival rates. Baseline characteristics were analyzed using the Wilcoxon test to assess the comparability of the groups with respect to factors possibly related to the outcome. Categorical variables were analyzed using Fisher's exact test. Student's paired *t*-test was used for comparisons between day 0 and day 2 SOFA scores in each group. To analyze the difference in the mortality, Kaplan–Meier survival curves were constructed for each study group and then compared using the log-rank test. A *P*-value of less than 0.05 was considered significant.

RESULTS

During the study period, 22 consecutive patients fulfilled the above inclusion criteria. Fourteen patients were eligible for treatment with rTM (rTM group), and eight patients who were treated without rTM were nominated as the historical controls (control group). The average of rTM administration period was 8.6 days. Baseline characteristics and therapeutic interventions of the study population are listed in Table 2. The numbers who developed septic shock due to operative complication were 8 out of 14 in the rTM group and 5 out of 8 in the control group, respectively. There was no significant difference in age or the severity of illness, as indicated by SOFA score. There was no difference in the site of infection between the groups. There was no significant difference in therapeutic interventions (CHDF or AT) between groups. PMX-DHP was done only once except in two cases in rTM group.

Major outcomes regarding survival rate are presented in Table 3 and Figure 1. Compared with the control group, the rTM group showed significantly better outcomes. The SOFA score at day 2 significantly improved in the rTM group (Fig. 2).

During the study period, one serious adverse event related to bleeding occurred only in the rTM group

TABLE 3. *Survival rate at 60 days*

	rTM group ($N = 14$)	Control group ($N = 8$)	P
N (%)	12 (85.7)	3 (37.5)	0.015

rTM, recombinant thrombomodulin.

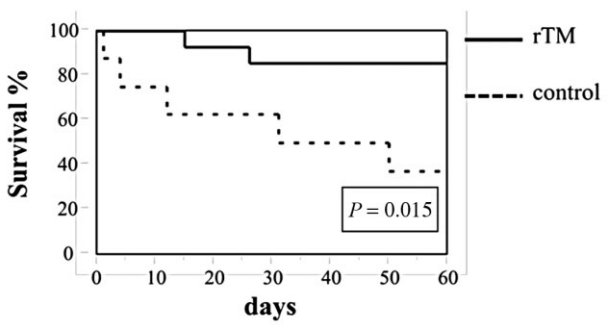

FIG. 1. Kaplan–Meier plot of the survival. rTM, recombinant thrombomodulin.

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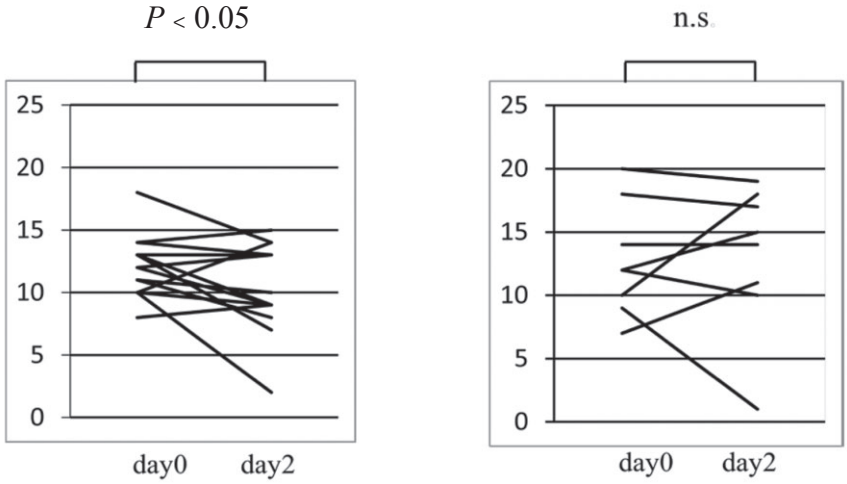

FIG. 2. The Sequential Organ Failure Assessment (SOFA) score at day 0 and 2. rTM, recombinant thrombomodulin.

(7.1%). The bleeding event was multiple cerebral hemorrhage on day 10 during rTM administration. The causal relationship between rTM and hemorrhage was possible, yet unclear.

DISCUSSION

In this study, we obtained a better survival rate in patients with the combination of PMX-DHP and rTM. We also found that rTM administration significantly improved total SOFA score, which suggests that the progress of organ dysfunction was prevented. We speculate how our treatment strategy works in sepsis-induced DIC (Fig. 3). In the septic state, DIC and multiple organ failure develop via the crosstalk of inflammation and coagulation with HMGB1 as its core (5). It is no exaggeration to say that not only an improvement in hemodynamics but also regulation of HMGB1 is the key factor for life prognosis of septic shock patients. Kobayashi et al. reported that in septic shock patients receiving PMX-DHP, HMGB1 was almost undetectable on the next day of PMX-DHP in the survival group, but elevated in the fatal group (14,15). This seems to suggest that PMX alone is insufficient to control HMGB1. Yamakawa et al. reported the utility of rTM for severe septic DIC cases requiring respiratory management with a ventilator (16). Kudo et al. reported that serum concentrations of HMGB1 decreased

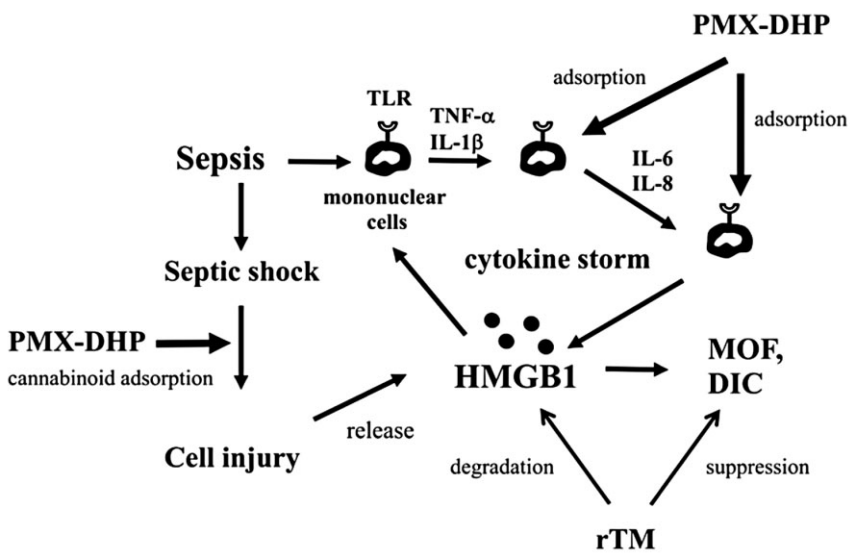

FIG. 3. The putative mechanism of the combination of Polymyxin-B direct hemoperfusion (PMX-DHP) and recombinant thrombomodulin (rTM) for septic shock accompanied by disseminated intravascular coagulation (DIC). HMGB1, High Mobility Group Box 1; TLR, toll-like receptor; MOF, multiple organ failure.

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within a couple of days after rTM administration in patients with sepsis-induced DIC (17). We have also experienced cases in which an increase in HMGB1 was seen despite a chronological decrease in IL-6 in patients who survived with combination therapy of PMX and rTM for septic shock complicated by DIC (18).

Optimal schedule of PMX-DHP and/or rTM administration remains to be elucidated. It has been reported that PMX-DHP improves the P/F ratio more when it is carried out longer than the conventional 2 h (19). We prefer long-hour application of PMX-DHP by monitoring not only hemodynamics but also respiratory conditions as the markers in cases with disturbed lung oxygenation at the introduction of PMX. In the present study the PMX duration in the rTM group was statistically longer than that in the control group. However, when the surviving and fatal patients are compared, there was no significant difference of the PMX-DHP duration between the surviving ($N = 15$) and the fatal patients ($N = 7$) (data not shown). Therefore, we think that the difference of survival rate between the control group and the rTM group was not simply due to PMX-duration.

We recognize that there are some limitations in this study. First, this is a retrospective historical controlled study and not a randomized controlled study. Second, this is a single-center study. Third, the sample size was small.

CONCLUSION

The combination of polymyxin-B direct hemoperfusion and recombinant thrombomodulin may improve the survival rate of septic shock cases with disseminated intravascular coagulation.

Disclosure: The authors declare no conflicts of interest.

REFERENCES

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;20:864–74.
2. Takeuchi O, Hoshino K, Kawai T et al. Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components. *Immunity* 1999;11: 443–51.
3. Wang H, Bloom O, Zhang M et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–51.
4. Wang H, Yang H, Tracey KJ et al. Extracellular role of HMGB1 in inflammation and sepsis. *J Inter Med* 2004;255: 320–31.
5. Hatada T, Wada H, Nobori T et al. Plasma concentrations and importance of High Mobility Group Box protein in the prognosis of organ failure in patients with disseminated intravascular coagulation. *Thromb Haemost* 2005;94:975–9.
6. Vincent JL, Laterre PF, Cohen J et al. A pilot-controlled study of a polymyxin B-immobilized hemoperfusion cartridge in patients with severe sepsis secondary to intra-abdominal infection. *Shock* 2005;23:400–5.
7. Cruz DN, Antonelli M, Fumagalli R et al. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. *JAMA* 2009;301:2445–52.
8. Wang Y, Liu Y, Sarker KP et al. Polymyxin B binds to anandamide and inhibits its cytotoxic effect. *FABS Lett* 2000;470:151–5.
9. Kobayashi M. Effects of Direct Hemoperfusion with Polymyxin B Immobilized Fiber (PMX-DHP) for Patients with Septic Multiple Organ Failure. *Jpn Apheresis* 2010;29: 266–71.
10. Nishibori M, Takahashi H, Katayama H et al. Specific removal of monocytes from peripheral blood of septic patients by polymyxin B-immobilized filter column. *Acta Med Okayama* 2009;63:65–9.
11. Ito T, Kawahara K, Okamoto K et al. Proteolytic cleavage of high mobility group box 1 protein by thrombin-thrombomodulin complexes. *Arterioscler Thromb Vasc Biol* 2008;10:1825–30.
12. Abeyama K, Stern DM, Ito Y et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel anti-inflammatory mechanism. *J Clin Invest* 2005;115: 1267–74.
13. Gando S, Iba T, Eguchi Y et al. A multicenter, prospective validation of disseminated intravascular coagulation diagnostic criteria for critically ill patients: comparing current criteria. *Crit Care Med* 2006;34:625–31.
14. Kobayashi M. Effects of direct hemoperfusion with polymyxin B immobilized fiber (PMX-DHP) on septic multiple organ failure. *J Abdomin Emerg Med* 2007;27:45–9.
15. Kobayashi M. Effects of PMX-DHP treatment for patients with septic acute lung injury/acute respiratory distress syndrome. *ICU & CCU* 2009;33:119–25.
16. Ogawa Y, Yamakawa K, Ogura H et al. Recombinant human soluble thrombomodulin improves mortality and respiratory dysfunction in patients with severe sepsis. *J Trauma* 2012;72: 1150–7.
17. Kudo D, Shinozawa Y, Yamanouchi S et al. Treatment effect of thrombomodulin-α on septic disseminated intravascular coagulation (DIC): a historical cohort study. *J Jpn Soc Intensive Care Med* 2012;19:359–66.
18. Yamato M, Minematsu Y, Kuroiwa T et al. Three Septic Cases: putative Role of a Combination of Polymyxin-B Direct Hemoperfusion and Recombinant Thrombomodulin for Treating Septic Shock Accompanied by Disseminated Intravascular Coagulation. *Jpn J Crit Care Endotoxemia* 2012; 16:148–53.
19. Mitaka C, Tsuchida N, Kawada K et al. A longer duration of polymyxin B-immobilized fiber column hemoperfusion improves pulmonary oxygenation in patients with septic shock. *Shock* 2009;32:478–83.

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