A Novel Strategy for Hemolytic Uremic Syndrome: Successful Treatment With Thrombomodulin α

abstract

Hemolytic uremic syndrome (HUS) is a life-threatening infectious disease in childhood for which there is no confirmed therapeutic strategy. Endothelial inflammation leading to microthrombosis formation via complement activation is the main pathology of HUS. Thrombomodulin is an endothelial membrane protein that has anticoagulation and anti-inflammatory effects, including the suppression of complement activity. Recombinant human soluble thrombomodulin (rTM) is a novel therapeutic medicine for disseminated intravascular coagulation. We administered rTM to 3 patients with HUS for 7 days and investigated the outcomes in view of the patients’ prognoses, changes in biochemical markers, complications, and adverse effects of rTM. Symptoms and laboratory data improved after initiation of rTM in all 3 patients. Abnormal activation of complements was also dramatically suppressed in 1 patient. The patients recovered without any complications or adverse effects of rTM. They were discharged having normal neurologic status and with no renal dysfunction. To our knowledge, this is the first report of rTM being used to treat HUS. These case reports show the positive effect of rTM in patients with HUS. Randomized controlled studies should be performed to assess the efficacy and safety of rTM for children with HUS. Pediatrics 2013;131:e1–e6

AUTHORS: Takashi Honda, MD, Shohei Ogata, MD, Eri Mineo, MD, Yukako Nagamori, MD, Shinya Nakamura, MD, Yuki Bando, MD, and Masahiro Ishii, MD

Department of Pediatrics, Kitasato University School of Medicine, Sagamihara, Japan

KEY WORDS
anticoagulation, anti-inflammation, complement, hemolytic uremic syndrome (HUS), thrombomodulin

ABBREVIATIONS
ARF—acute renal failure
CHDF—continuous hemodiafiltration
DIC—disseminated intravascular coagulation
Hb—hemoglobin
HUS—hemolytic uremic syndrome
LDH—lactate dehydrogenase
rTM—recombinant human soluble thrombomodulin
SUN—serum urea nitrogen

Dr Honda conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted; Dr Ogata conceptualized and designed the study, reviewed and revised the manuscript, and approved the final manuscript as submitted; Drs Mineo, Nagamori, and Nakamura conducted the initial analyses, critically reviewed the manuscript, and approved the final manuscript as submitted; Drs Bando conducted the final analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted; and Dr Ishii conceptualized and designed the study, reviewed and revised the manuscript, and approved the final manuscript as submitted.

This trial has been registered by the ethics board of Kitasato University Hospital (C09-536).

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Address correspondence to Masahiro Ishii, MD, Department of Pediatrics, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0329, Japan. E-mail: ishimm@med.kitasato-u.ac.jp

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Hemolytic uremic syndrome (HUS) is a life-threatening infectious disease characterized by 3 symptoms: microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (ARF). Although outbreaks of Escherichia coli infection due to eating raw or not well-cooked meat have reportedly led to a large number of patients developing HUS, supportive therapy including dialysis is still the most effective treatment. It was recently reported that plasma exchange could reduce mortality; however, this treatment remains controversial. Therefore, establishing a specific confirmed therapy for HUS is necessary.

The main pathology of HUS is endothelial inflammation leading to activated coagulation, which causes the formation of fibrin thrombosis resulting in the 3 characteristic symptoms. Recombinant human soluble thrombomodulin (rTM) is the first medicine comprising the extracellular domain of human thrombomodulin, and it exerts anticoagulation and anti-inflammatory effects on endothelial cells in several ways. It was recently reported that rTM would be effective for HUS as an anticoagulant and anti-inflammatory medicine. To our knowledge, this is the first clinical report of rTM administration being used to suppress the progression of endothelial inflammation and the subsequent thrombogenesis in HUS.

### PATIENT PRESENTATION

Three patients were diagnosed as having typical HUS based on the following diagnostic criteria: (1) occurrence after an acute gastrointestinal illness; (2) anemia with microangiopathic changes; (3) renal injury evidenced by either hematuria, proteinuria, or elevated creatinine level; and (4) thrombocytopenia (platelets <150,000/μL). The patients had no family history of HUS, medical history of renal dysfunction, or bowel diseases. The patients’ characteristics and clinical data are summarized in Table 1. All patients were administered 380 U/kg of rTM per day. The ethics board of Kitasato University Hospital approved the therapeutic use of rTM, and we obtained informed written consent from the parents of each patient.

#### Patient 1

A 1.8-year-old girl was admitted to our hospital after a 3-day history of diarrhea, bloody stool, and vomiting. She had eaten insufficiently cooked beef. She was delirious (Glasgow Coma Scale E4, V3, M5) and looked pale. Edema was observed. Laboratory data showed the following: hemoglobin (Hb), 9.1 g/dL; platelets, 2.7 × 10^9/μL; lactate dehydrogenase (LDH), 2693 U/L; serum urea nitrogen (SUN), 100.8 mg/dL; and creatinine, 2.15 mg/dL. Results of a stool culture test revealed the existence of E. coli (O111). We diagnosed the patient as having HUS and started administration of rTM. Continuous hemodiafiltration (CHDF) was performed because of ARF, and blood transfusions were repeated as needed.

After starting rTM and CHDF, the frequency of blood transfusions gradually decreased, and LDH levels decreased (Fig 1A). Hb and platelet levels started increasing, and creatinine decreased. D-dimer decreased after recording a peak on day 8. We also examined the changes in complements (Fig 1B). The level of C3 dramatically increased from 62 to 138 mg/dL after administration of rTM. The level of C4 also quickly increased from 13 to 31 mg/dL. Bb and SC5b-9 peaked on days 5 and 9, respectively, and subsequently decreased.

On day 13, we succeeded in weaning the patient from CHDF. The patient recovered consciousness after 17 days, with no adverse effects from rTM. Although a transient elevation of creatinine was recorded after weaning CHDF, creatinine levels returned to a normal level on day 18.

#### Patient 2

A 10-year-old girl was hospitalized after a 6-day history of diarrhea and stomach ache. She had eaten medium rare beef with her family 3 days before the onset of her abdominal symptoms. Her father, mother, brother, and sister also suffered from abdominal symptoms. The patient was conscious but looked

### Table 1 Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient No.</th>
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<tr>
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<td>1</td>
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<tr>
<td>Age, y</td>
<td>1.8</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Chief complaint</td>
<td>Diarrhea, bloody stool, vomiting</td>
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<tr>
<td>Neurologic status (Glasgow Coma Scale)</td>
<td>Delirious (E4, V3, M5)</td>
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<tr>
<td>Bacteria (type)</td>
<td>E. coli (O111)</td>
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<tr>
<td>Illness days on admission</td>
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<tr>
<td>Illness days at initiation of rTM</td>
<td>3</td>
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<tr>
<td>CHDF</td>
<td>yes</td>
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<tr>
<td>Blood transfusion</td>
<td>yes</td>
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slightly pale. Laboratory data showed the following: Hb, 9.8 g/dL; platelets, $2.2 \times 10^4/\mu L$; LDH, 2740 U/L; SUN, 92.7 mg/dL; and creatinine, 2.12 mg/dL. Although her stool culture showed no bacteria, stool samples from her family revealed *E coli* (O157). We diagnosed her as having HUS and started rTM; she was given a platelet transfusion on day 6. The patient’s Hb and platelet levels gradually increased, and LDH and D-dimer levels decreased (Fig 2). Creatinine also started decreasing, and her diarrhea and stomach ache ceased on day 8. The patient recovered without any complications or adverse effects.

**Patient 3**

A 2-year-old boy was hospitalized after a 4-day history of diarrhea, bloody stool, vomiting, and stomach ache. His father also had diarrhea. The patient was confused (Glasgow Coma Scale E4, V4, M6), and looked pale. Edema was not detected. Laboratory data revealed the following: Hb, 10.3 g/dL; platelets, $5.1 \times 10^4/\mu L$; LDH, 1132 IU/L; SUN, 30.4 g/dL; and creatinine, 0.76 mg/dL. Urinalysis showed hematuria and proteinuria. Results of his stool culture showed *E coli* (O157). On day 5, LDH, D-dimer, and creatinine levels decreased slightly; however, the patient remained confused, and we therefore started rTM therapy. On day 6, he became alert. Hb and platelet levels gradually increased, while LDH and D-dimer levels decreased (Fig 3). The amount of urine increased, and creatinine decreased. The patient recovered without any complications or adverse effects.

**DISCUSSION**

In HUS, a toxin’s direct damage to endothelial cells and the adhesion of neutrophils and monocytes via nuclear factor κB cause endothelial damage and inflammation.6,7 Hemolytic anemia is the result of mechanical damage to red blood cells as they pass through the altered vasculature. The resulting thrombogenesis on the damaged endothelial cells promotes coagulation and consumption of platelets and coagulation factors, resulting in thrombocytopenia and bleeding symptoms. Thrombomodulin blocks adhesion of neutrophils to endothelial cells by

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**FIGURE 1**

A, Changes in the laboratory data of patient 1. Upper panel, changes in LDH, D-dimer, and platelets normalized after initiation of rTM. Lower panel, creatinine decreased and the amount of urine increased. Transient elevation of creatinine was recorded after CHDF weaning, and creatinine returned to a normal level on day 18. B, Changes in complement proteins. Upper panel, C3 and C4 dramatically increased after initiation of rTM. Lower panel, Bb and SC5b-9 decreased. In normal plasma, the Bb and SC5b9 concentrations are 1195 ± 583 and 402 ± 167 ng/mL, respectively.
interference with nuclear factor κB pathways. In addition, thrombomodulin activates protein C, and activated protein C regulates excess coagulation. We conjectured that regulation of inflammation and thrombogenesis of endothelial cells is an essential therapeutic goal for patients diagnosed with HUS because they are the main pathologies in this infectious disease. Zoja et al demonstrated that mice whose thrombomodulin function had been impaired exhibited more severe symptoms and shortened survival, with stronger inflammatory reaction in the kidney and more abundant fibrin in the brain. Their study strongly supports our hypothesis that use of rTM could be a novel and groundbreaking treatment strategy for patients with HUS.

Saito et al and Yamakawa et al reported the clinical effect of rTM for DIC treatment. Although HUS is different from DIC in that fibrinolysis is suppressed, endothelial inflammation and activated coagulation, which could be the therapeutic targets of rTM, are common to both HUS and DIC. Elevated D-dimer and fibrin degradation products in the 3 patients in the current study reflect such pathophysiology.

We assessed D-dimer as an index of coagulation, and its decrease reflected the efficacy of rTM in suppressing coagulation. Patient 1’s condition was extremely severe, and many thrombi had already formed on initiation of rTM. D-dimer levels do not reflect activity of thrombogenesis but the existence of thrombosis. We considered, therefore, that the patient had recorded highly elevated D-dimer levels and transient increases just after initiation of rTM. We also noted that a dramatic decrease in LDH reflects both relief of hemolytic anemia and recovery of microcirculation brought on by inhibition of thrombosis in multiple systemic organs. In patient 3, although D-dimer and LDH levels slightly decreased before initiation of rTM treatment, the patient’s neurologic status recovered and laboratory data never deteriorated after rTM initiation. These prognoses and laboratory changes in these 3 patients led us to conclude that rTM was effective for the treatment of HUS.

**FIGURE 2**
Changes in the laboratory data of patient 2. Upper panel, changes in LDH, D-dimer, and platelets normalized after initiation of rTM. Lower panel, creatinine decreased, while the amount of urine increased.

**FIGURE 3**
Changes in the laboratory data of patient 3. Upper panel, LDH, D-dimer, and platelets normalized after initiation of rTM. Lower panel, creatinine decreased, while the amount of urine increased.
The activation of complements has been shown to play an important role in endothelial inflammation.6–8 The positive effect of eculizumab, which is a humanized antibody against complement C5 and a recognized therapy for paroxysmal nocturnal hemoglobinuria and atypical HUS, was reported also in patients with diarrhea-associated HUS.18 rTM also has the effect of blocking complement pathways.12,19 Therefore, we analyzed the changes in the complements in patient 1. After starting rTM, C3 and C4 dramatically increased, and Bb and SC5b-9 decreased. Judging from these changes, rTM suppressed alternative pathways of the complements, leading to increases in C3 and C4 and a decrease in Bb, which is an indicator of alternative pathways; subsequently, SC5b-9, which is the end product of complement cascade, started decreasing.8 Although these data cannot fully clarify the effect of rTM on complements because we investigated changes in complements in only 1 case, they are consistent with the pharmacologic mechanism of rTM.

All 3 patients were discharged with normal neurologic status and with normal renal function. Because neurologic involvement and ARF are the consequences of endothelial inflammation and microthrombosis in the brain and kidney,5,6,20 we posit that the anti-inflammatory and anti-coagulation effects of rTM affected them significantly. Therefore, we propose that rTM contributed to the prevention and recovery from neurologic involvement and ARF. No bleeding symptoms were observed in any of the patients in the current study. rTM has been reported to alleviate bleeding symptoms better than heparin therapy in patients with DIC.14 rTM has also been shown to have a wide safety margin and a favorable antithrombotic profile.21 No other adverse effects were observed in these patients.

A limitation of this study is that it was observational and lacking a control treatment arm. Another limitation is that the pharmacokinetical monitoring of each patient was not evaluated. In addition, we did not establish or assess the criteria for rTM initiation. However, we theorized that biomarkers such as D-dimer and the neurologic status are possible criteria because elevated D-dimer levels and encephalopathy reportedly lead to worse prognoses.20,22 A larger randomized trial is warranted to verify the efficacy and safety of rTM for children with HUS and to determine the type of HUS patients for whom rTM should be initiated.

CONCLUSIONS

We administered rTM to 3 patients with HUS, all of whom recovered without any complications or adverse effects. We propose that using rTM is a novel and promising therapeutic approach to treating children with HUS. This case report, of 3 pediatric patients, paves the way for larger randomized controlled trials to verify the efficacy and safety of rTM for children with HUS.

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REFERENCES


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