The efficacy of recombinant human soluble thrombomodulin for the treatment of shiga toxin-associated hemolytic uremic syndrome model mice

Kazuhide Suyama, Yukihiro Kawasaki, Kyohei Miyazaki, Syuto Kanno, Atsushi Ono, Shinichiro Ohara, Masatoki Sato and Mitsuaki Hosoya

Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima City, Fukushima, Japan

Correspondence and offprint requests to: Yukihiro Kawasaki; E-mail: kyuki@fmu.ac.jp

ABSTRACT

Background. Recombinant human soluble thrombomodulin (rhTM) is a promising therapeutic natural anticoagulant that is comparable to antithrombin, tissue factor pathway inhibitor and activated protein C. In order to clarify the efficacy of rhTM for the treatment of typical hemolytic uremic syndrome (t-HUS), we examined changes in renal damage in t-HUS mice treated with rhTM or vehicle alone.

Methods. We used severe and moderate t-HUS mice injected with shiga toxin (Stx) and lipopolysaccharide (LPS). The severe t-HUS mice were divided into two subgroups [an rhTM subgroup (Group A) and a saline subgroup (Group B)] along with the moderate t-HUS mice [an rhTM subgroup (Group C) and a saline subgroup (Group D)]. Groups E and F were healthy mice treated with rhTM or saline, respectively.

Results. All mice in Group B died at 80–90 h post-administration of Stx2 and LPS whereas all mice in Group A remained alive. Loss of body weight, serum creatinine level, endothelial injury and mesangiolysis scores at 24 h after administration in the t-HUS mice treated with rhTM were lower than those in t-HUS mice treated with saline. The levels of hemoglobin at 6 h and platelet counts at 24 h after administration in Group A were higher than those in Group B. Serum interleukin (IL)-6, IL-1β and chemical hypoxia and ischemia/reperfusion injury by inhibition of the mitochondrial calcium uniporter. Toxicol Appl Pharmacol 2013; 273: 172–179


41. Ong SB, Hall AR, Dongworth RK et al. Akt protects the heart against ischemia–reperfusion injury by modulating mitochondrial morphology. Thromb Haemost 2014; 113 (Epub ahead of print)

Received for publication: 25.9.2014; Accepted in revised form: 11.12.2014
tumor necrotic factor (TNF)-α levels at 24 h after administration in Group A were lower than those in Group B. Serum C₅b₉ levels at 24 h after the administration and serum fibrinogen degradation product (FDP) at 72 h after the administration of Stx2 and LPS were lower in Group A than in Group B.

Conclusions. These results indicate that rhTM might afford an efficacious treatment for t-HUS model mice via the inhibition of further thrombin formation and amelioration of hypercoagulant status.

Keywords: hemolytic uremic syndrome, lipopolysaccharide, mice, recombinant human soluble thrombomodulin, verotoxin

INTRODUCTION

Typical hemolytic uremic syndrome (t-HUS), defined as a triad of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure, is a renal disease associated with endothelial cell dysfunction. Electron microscopy (EM) examination shows endothelial swelling and detachment from the basement membrane [1–4], while the pathological condition is similar to that of kidneys with disseminated intravascular coagulation (DIC). The cause is shiga toxin (Stx)-producing *Escherichia coli* (STEC) infection in most infant cases, and t-HUS is the most common cause of acute renal failure in children [2, 3]. Large-scale outbreaks of t-HUS have recently been reported in Japan, northern Germany and 15 other countries in Europe [4].

T-HUS mortality is reported to be between 3 and 5%, and deaths due to t-HUS are nearly always associated with severe extrarenal disease that includes severe central nervous system involvement. Approximately two-thirds of children with t-HUS require dialysis therapy, whereas the remaining one-third experience milder renal involvement without the need for dialysis therapy [1–8]. As to the treatment of t-HUS, to date, most t-HUS patients have received supportive treatment. However, there are currently a few specific treatments including therapeutic plasma exchange and/or eculizumab, a humanized monoclonal antibody that inhibits the C5 terminal complement common pathway [9, 10].

On the other hand, thrombomodulin (TM) is a transmembrane protein displayed on the endothelial cell surface that plays an important role in the regulation of intravascular coagulation [11, 12]. Recombinant human soluble thrombomodulin (rhTM) binds to thrombin to inactivate coagulation, and the thrombin–rhTM complex subsequently activates protein C to produce activated protein C (APC), which, in the presence of protein S, inactivates factors VIIIa and Va, thereby inhibiting further thrombin formation [13]. Thus, rhTM might afford a beneficial treatment for sepsis patients with reduced endothelial TM, and it is currently being used clinically for the treatment of DIC in Japan [14]. Recently, Honda *et al.* reported a positive effect from the use of rhTM in patients with t-HUS [15, 16]. Thus, it is speculated that rhTM could become a specific treatment for patients with t-HUS. However, there have been no detailed reports on the mechanism underlying the efficacy of rhTM for t-HUS.

With regard to t-HUS models, there have been some reports on a mouse model of t-HUS induced by the coinjection of Stx2 and lipopolysaccharide (LPS) [17–19]. In order to clarify the efficacy of rhTM for t-HUS, we used severe and mild t-HUS model mice and examined changes in renal damage in t-HUS mice treated with rhTM or vehicle alone.

MATERIALS AND METHODS

Disease model

Animal experiments were performed using 5-week-old male inbred C57BL/6 mice (Japan SLC, Inc., Shizuoka, Japan). Mice were allowed free access to normal mice chow and tap water. All animal experiments were performed according to the Institutional Animal Care and Use Committee guidelines of Fukushima Medical University School of Medicine (FMUSM).

Stx and LPS

Stx2 was produced in *E. coli* DH5 strain using the pLPSh3 plasmid and purified by immunoaffinity chromatography. LPS (O55:B5), purified by gel filtration chromatography and gamma irradiation, was purchased from Sigma-Aldrich (St. Louis, MO).

rhTM

rhTM, composed of the active, extracellular domain of TM, was provided by Asahi-Kasei Pharma Corporation. The dose of rhTM (380 IU/kg) was similar to the rhTM dose used for adult human patients with DIC.

Experimental protocol

We used severe t-HUS and mild t-HUS mice as a t-HUS model. Severe t-HUS mice received intraperitoneal injections of Stx2 at 225 ng/kg, and LPS at 300 μg/kg, while moderate t-HUS mice received intraperitoneal injection of Stx2 at 50 ng/kg, and LPS at 100 μg/kg. The severe t-HUS mice (n = 30) were divided into two subgroups [an rhTM subgroup (Group A) and a saline subgroup (Group B)] along with the moderate t-HUS mice (n = 40) [an rhTM subgroup (Group C) and a saline subgroup (Group D)]. Each rhTM subgroup consisted of mice treated with rhTM (380 IU/kg) at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS. Each saline subgroup consisted of mice treated with saline at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS. Group E was a healthy animal group consisting of mice treated with rhTM at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS. Group E was a healthy animal group consisting of mice treated with rhTM at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS. Each saline subgroup consisted of mice treated with saline at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS. Each saline subgroup consisted of mice treated with saline at 6 h before, at the time of administration and at 6 h after the administration of Stx and LPS.
Laboratory investigation

All blood analyses were performed at the FMUSM Laboratories. Mice were euthanized and whole blood was collected in hematology tubes containing tripotassium ethylenediaminetetraacetic acid for cell count analysis. Furthermore, whole blood was left to coagulate at room temperature for 10 min after which samples were centrifuged at 3000 r.p.m. at 4°C for 10 min and serum was collected. Serum creatinine (Cr) levels, serum cytokines, chemokines, C5b-9 levels and fibrin/FDPs were then determined.

Histological examination

LM, IHM, EM. The renal tissue was fixed in buffered formalin and embedded in paraffin for light-microscopic examination. Sections (2- to 3-μm in thickness) were then individually stained with hematoxylin–eosin, periodic acid–Schiff and periodic acid–silver methenamine, and observed under a light microscope. Three observers semiquantitatively graded extracellular matrix accumulation in each quadrant in 20 glomeruli per kidney on a scale from 0 to 3 as follows: endothelial injury score: 0 = absence of mesangiolysis, 1 = mesangial area (MA) exhibiting slight lucency [0–25% disruption of mesangial cell (MC)], 2 = MA exhibiting moderate lucency (25–50% disruption of MC) with preservation of the underlying glomerular tuft architecture and 3 = MA exhibiting marked lucency (50–100%) with degeneration and disruption of MC, usually in association with microaneurysm formation; mesangial cell proliferation score: 0 = absence of mesangial cell proliferation, 1 = slight increase in mesangial cells, 2 = moderate increase in mesangial cells and 3 = marked increase in mesangial cells.

Immunoperoxidase staining for α-smooth muscle actin (SMA) and CD31 were evaluated by the methods previously described by Kawasaki et al. [20]. Mouse anti-human α-SMA (1A4; Dako, Glostrup, Denmark) and rabbit anti-mouse CD31 (ER-MP12; BMA) polyclonal antibodies were used as the primary antibodies for IHM.

Multiplex analysis of cytokine and chemokines protein expression and coagulant findings in mice

Luminex analysis was performed for G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, interleukin (IL)-10, IL-12, IL-13, IL-15, IL-17, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IP-10, macrophage chemoattraction protein-1, macrophage inflammatory protein-1α, regulated upon activation, normal T cell expressed and secreted (RANTES) and tumor necrotic factor (TNF)-α in the sera samples taken from the mice. Cytokine and chemokine concentrations were determined using the Mouse 32-Plex cytokine/chemokine panel bead immunoassay. The serum FDP and C5b-9 (MAC) in samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for Terminal Complement Complex C5b-9 [Mus musculus (Mouse), Uscn, Life Science Inc., China] or (ELISA) kit for FDP [Mus musculus (Mouse), Uscn, Life Science Inc., China].

Statistics

Values are shown as the mean values ± SD. Statistical analyses were performed using Stat View software (Abacus Concepts, Berkeley, CA, USA). Differences in laboratory data among groups were assessed using a non-parametric test, such as Mann–Whitney’s rank-sum test or the Kruskal–Wallis test. Correlations were evaluated using Fisher’s r-test. Findings of P ≤ 0.05 were considered significant.

RESULTS

Comparison of body weight and renal findings among groups

The ratio of body weight loss at 72 h after the administration of Stx2 and LPS in Group A was lower than that in Group B (6.2 ± 1.7% versus 12.9 ± 2.0%, P < 0.01) (Figure 1). The ratio of body weight loss in Group C was lower than that in Group D (1.9 ± 1.3% versus 6.8 ± 1.9%, P < 0.01). Subsequently, the ratio of body weight loss decreased and body weights at 14 days after the administration of Stx2 and LPS in Group A and Group B were the same as those before administration. The ratio of body weight gain at 14 days after administration of Stx2 and LPS in Group C were higher than those in Group D (4.0 ± 1.5% versus −0.8 ± 1.6%, P < 0.01). Fragmented erythrocytes were found in mice of all groups at 6 h after the administration of Stx2 and LPS. The platelet counts (×104/μL) at 24 h in Group A and Group B were lower than that in Group F (39.6 ± 7.3 versus 130.1 ± 4.6, P < 0.01, and 24.9 ± 8.1 versus 130.1 ± 4.6, P < 0.01, respectively). The platelet counts at 24 h in Group A were higher than that in Group B (39.6 ± 7.3 versus 24.9 ± 8.1, P < 0.05). The levels of hemoglobin (×104/μL) at 6 h in Group A and Group B were lower than that in Group F (40.4 ± 2.9 versus 47.1 ± 1.2, P < 0.01, and 34.4 ± 3.2 versus 47.1 ± 1.2, P < 0.01, respectively). The levels of hemoglobin at 6 h in Group A were higher than that in Group B (40.4 ± 2.9 versus 34.4 ± 3.2, P < 0.05). The serum Cr level (mg/dL) at 72 h after the administration of Stx2 and LPS in Group A was lower than that in Group B (1.3 ± 0.3 versus 3.6 ± 0.4, P < 0.01). The serum creatinine level (mg/dL) at 72 h after injury in Group C was also lower than that in Group D (1.2 ± 0.2 versus 2.4 ± 0.3, P < 0.01).

Subsequently, all mice in Group B died at 80–90 h post-administration of Stx2 and LPS whereas all mice in Group A remained alive. The serum creatinine levels in Group A, Group C and Group D peaked from 72 h to 7 days after injury and decreased at 14 days after the administration of Stx2 and LPS. The serum creatinine level at 7 days after the administration of Stx2 and LPS in Group C was lower than that in Group D (1.2 ± 0.3 versus 1.5 ± 0.3, P < 0.01). No significant differences were observed in the ratio of body weight gain, platelet counts, levels of hemoglobin or serum creatinine between Group E and Group F.

Intergroup comparisons of light-microscopic and immunohistopathological findings with time after the administration of Stx2 and LPS

In Group B, the endothelial injury score at 72 h was higher than that at 24 h (3.0 ± 0.2 versus 2.3 ± 0.3, P < 0.05) (Figure 2a). In the other groups, the endothelial injury scores at 24 h were higher than those at 72 h, 7 days and 14 days, with the endothelial injury scores gradually decreasing with time. Endothelial injury scores at 24 and 72 h in Group A were lower than those in
Group B (1.7 ± 0.4 versus 2.3 ± 0.3, P < 0.05, and 1.2 ± 0.2 versus 2.8 ± 0.3, P < 0.01, respectively). In addition, the endothelial injury score at 24 h in Group C was lower than that in Group D (1.0 ± 0.2 versus 1.7 ± 0.3, P < 0.05).

Mesangial proliferation scores at 24 and 72 h in Group B were low (1.3 ± 0.2 versus 0.9 ± 0.2, respectively) (Figure 2b), with that at 72 h in Group A higher than that in Group B (1.6 ± 0.5 versus 0.9 ± 0.2, P < 0.01). There was no difference in mesangial proliferation scores at 72 h between Group C and Group D (1.4 ± 0.4 versus 1.8 ± 0.2, respectively), but mesangial proliferation scores from 7 days in Group C decreased whereas those in Group D gradually increased. The mesangial proliferation score at 7 days in Group D was higher than that in Group C (2.0 ± 0.4 versus 1.0 ± 0.3, P < 0.01).

In Group B, the glomerular CD31 staining scores at 24 and 72 h were very low (0.8 ± 0.1 versus 0.4 ± 0.1, respectively) (Figures 2C and 3), with those at 24 and 72 h in Group A higher than those in Group B (1.3 ± 0.4 versus 0.4 ± 0.1, P < 0.01, and 1.1 ± 0.2 versus 0.8 ± 0.1, P < 0.05, respectively).

Comparison of electron-microscopic findings between group A and group B

Endothelial cell injury and mesangiolysis were apparent in Group A and Group B from 24 to 72 h (Figure 4). In Group B, severe mesangiolysis, the degeneration of endothelial cells and cystic dilatation of the glomerular tuft and abnormal hemocoagulation were found at 72 h after the administration of Stx2 and LPS. In Group A, mild mesangiolysis, degeneration of endothelial cells and cystic dilatation of the glomerular tuft were observed at 24 h after the administration of Stx2 and LPS. Mesangial cell proliferation was subsequently observed from 7 to 14 days.

Intergroup comparison of cytokine and chemokine levels together with coagulant findings over time after the administration of Stx2 and LPS

Serum IL-6, IL-1β, TNF-α and MCP-1 levels at 24 and 72 h in each group are shown in Figure 3. Serum IL-6 levels at 24 h in Group A and Group B were higher than that in Group F (146 ± 30 versus 95 ± 3, P < 0.05, and 189 ± 28 versus 95 ± 3, P < 0.05, respectively) and the serum IL-6 level at 24 h in Group A was lower than that in Group B (146 ± 30 versus 189 ± 28, P < 0.05). The serum IL-6 level at 24 h in Group A was lower than that in Group B (97 ± 6 versus 151 ± 41, P < 0.01), and the serum IL-6 level at 72 h in Group A was similar to that in Group F (97 ± 6 versus 95 ± 2, respectively). The serum IL-1β level at 24 h in Group B was higher than that in Group F (63 ± 23 versus 0 ± 0, P < 0.01, respectively), and the serum IL-1β level at 24 h in Group A was lower than that in Group B (20 ± 28 versus 63 ± 23, P < 0.05) (Figure 5).

Serum TNF-α levels at 24 h in Group A were lower than that in Group B (37.6 ± 3.7 versus 42.0 ± 3.7, P < 0.05). Serum MCP-1 levels at 24 h in Group A and Group B were higher than that in Group F (485 ± 130 versus 93 ± 25, P < 0.01, and 814 ± 346 versus 93 ± 25, P < 0.01, respectively), and there was no difference in the serum MCP-1 level at 24 h between Group A and Group B (814 ± 346 versus 93 ± 25, P = 0.08). Serum MCP-1 levels at 72 h in Group A and Group B were similar to that in Group F.

Serum FDP and serum C5b-9 levels for each group are shown in Figure 4. Serum FDP levels (ng/mL) at 72 h in Group A and Group B were higher than that in Group F (515 ± 195 versus 128 ± 94, P < 0.01, and 845 ± 309 versus 128 ± 94, P < 0.01, respectively), and the serum FDP level at 72 h in Group A was lower than that in Group B (515 ± 195 versus 845 ± 309, P < 0.05).

Serum C5b-9 levels (ng/mL) at 24 h in Group A and Group B were higher than that in Group F (515 ± 195 versus 128 ± 94, P < 0.01, and 845 ± 309 versus 128 ± 94, P < 0.01, respectively), and the serum FDP level at 72 h in Group A was lower than that in Group B (515 ± 195 versus 845 ± 309, P < 0.05).
DISCUSSION

Although there have been some reports on the t-HUS animal models used to investigate the pathological findings of t-HUS, no study has completely investigated the full pathophysiology of t-HUS. Keepers et al. established that C57BL/6 mice induced by LPS and Stx2 afford a model of t-HUS that includes the thrombocytopenia, hemolytic anemia and renal failure that define the disease in humans [17]. This mouse model is useful for the identification of therapeutic targets and development of new treatments for t-HUS. However, C57BL/6 mice treated
by LPS and Stx2 are insufficient for the evaluation of the recovery process from renal injury in t-HUS as the mice typically died at 3–4 days. Thus, we have developed a mild t-HUS model through treatment with a low, sublethal dose of Stx2 and LPS. These mild t-HUS model mice showed the same thrombocytopenia, hemolytic anemia and renal failure that define the disease in humans while surviving the administration of LPS and Stx2 [18].

As explained above, rhTM binds to thrombin to inactivate coagulation, and the thrombin–rhTM complex activates protein C to produce APC, which in the presence of protein S inactivates factors VIIIa and Va, thereby inhibiting further thrombin formation. rhTM has a long half-life (about 20 h) and has been shown to have a wider safety margin than other anticoagulants as well as having a favorable antithrombotic profile with less bleeding in animal and in vitro experiments [13]. Saito et al. have shown that rhTM therapy improves DIC and alleviates bleeding symptoms in DIC patients more significantly than does heparin therapy [14]. In addition, Kato et al. reported that rhTM might improve sepsis-induced DIC without an increased risk of bleeding. It has been shown that rhTM does not activate protein C after inhibiting thrombin generation, because rhTM exerts its anticoagulant effect in a thrombin-dependent manner [21]. Thus, it is speculated that rhTM could become a treatment for patients with t-HUS with reduced endothelial TM. However, there have been no detailed reports on the mechanism underlying the efficacy rhTM for t-HUS and no animal studies on t-HUS.

In our study, all severe t-HUS model mice treated with saline died at 80–90 h post-treatment whereas all severe t-HUS model mice treated with rhTM remained alive. The platelet counts in the severe model mice treated with rhTM were higher than those in the severe model mice treated with saline and the serum Cr in the model mice treated with rhTM was lower than that in model mice treated with saline. With regard to pathological findings, endothelial injury and mesangiolysis scores at 24 h in the t-HUS model mice treated with rhTM were lower than those in the t-HUS model mice treated with saline. Furthermore, CD31 expression at 72 h in the t-HUS model mice treated with rhTM was higher than those in t-HUS model mice treated with saline. T-HUS model mice treated with rhTM did not progress to renal failure.
Additionally, they showed signs of a recovery process, such as mesangial proliferation after renal injury. These results suggest that rhTM reduces both endothelial cell injury and renal dysfunction.

The mechanism underlying the effects of rhTM therapy for t-HUS is speculated to proceed as follows.

(i) With regard to the anti-coagulative effects of rhTM, the inhibition of this process acts via either the production of APC or the disruption of thrombin by the direct binding of rhTM, thus producing an anti-thrombin effect. TM is a transmembrane protein displayed on the endothelial cell surface, and the thrombin–TM complex activates protein C to produce APC [13]. In the presence of protein S, APC inactivates factors VIIIa and Va, thereby inhibiting further thrombin formation. In addition, TM is able to directly combine with thrombin, thereby resolving it. In our study, the serum FDP at 72 h after the administration of Stx2 and LPS was lower in the t-HUS model mice treated with rhTM than in those treated with saline. This result indicates that rhTM can improve hypercoagulative status in cases of t-HUS.

(ii) With regard to the anti-inflammatory effects of rhTM, it has been shown to inhibit the adhesion of neutrophils to vascular endothelia [22]. It had also been suggested that rhTM is an important molecule in various defense mechanisms, including the control of the coagulation system and suppression of inflammation [23]. Abeyama et al. found that rhTM inactivates a high-mobility group B1 DNA-binding protein, known as a lethal mediator during the late inflammation period, by binding and enhancing its proteolysis by thrombin [24, 25]. Furthermore, Shi et al. suggested that the lectin-like domain of rhTM binds to LPS and neutralizes LPS-induced inflammatory responses [26, 27]. Thus, rhTM is involved not only in...
anticoagulation but also in anti-inflammatory mechanisms and plays a central role in defense mechanisms involving endothelial cells. In our study, serum IL-6 and IL-1β levels as well as TNF-α levels at 24 h after the administration of Stx2 and LPS in t-HUS model mice treated with rhTM were lower than those in t-HUS model mice treated with saline. These findings indicate that rhTM inhibits inflammatory cytokines and chemokines in the pathological state of t-HUS, which is in agreement with the results of the reports cited above.

Furthermore, there have been a number of studies on the relationship between rhTM and vascular endothelial cell injury [24, 28, 29]. Ozaki et al. examined apoptotic cell death in in vitro experiments using human umbilical vein endothelial cells to study the effects of rhTM on the endothelial cell injury and found that rhTM significantly suppressed the number of apoptotic cells [28]. In addition, Conway et al. showed that rhTM protects vascular endothelial cells from serum deprivation-induced cell death. We speculate that the protection of endothelial cell by rhTN described in these studies is associated with our findings [29].

With regard to the complement in t-HUS model mice, Morigi et al. reported that alternative complement pathway activation by Stx promoted C5a formation, thus triggering microvascular thrombosis [22, 30]. On the other hand, C5b-9 as part of the membrane attack complex (MAC) is typically formed on the surface of intruding pathogenic bacterial cells as a result of the activation of the alternative pathway of the complement system, and it is one of the effector proteins of the immune system. MAC forms transmembrane channels [31] that disrupt the phospholipid bilayer of target cells, leading to cell lysis and death. In our study, the serum C5b-9 levels at 24 h after the administration of Stx2 and LPS were lower in the t-HUS model mice treated with rhTM than in the t-HUS model mice treated with saline. This finding suggested that rhTM inhibited complement activation. Van de Wouwer et al. also mentioned that the lectin-like domain of TM interferes with complement activation and protects against acute inflammatory arthritis [32]. Furthermore, Delmas et al. reported that eculizumab was effective for outbreak of t-HUS due to E. coli O104:H4 in France. Thus, rhTM may act to block the complement pathways.

One limitation to this study is that the t-HUS mouse model reported by Keepers is not so similar to human t-HUS as there is no Gb3 receptor present in the mouse glomerular endothelium. To further evaluate the efficacy of rhTM for t-HUS, we think that it is necessary to investigate the efficacy of rhTM for baboon t-HUS models that contain a Gb3 receptor in the glomerular endothelium [33]. Furthermore, as to the period of administration of rhTM, our treatment regimen was a combination of pre-/post-treatment. In a number of preliminary experiments using a post-treatment regimen, severe t-HUS mice were treated with rhTM either once at the time of Stx/LPS administration or once at the time of Stx/LPS administration and again 6 h after the challenge, but all mice died. We, therefore, believe that further study of the period and dose of rhTM administration as a post-treatment regimen for t-HUS models is necessary.

As to the efficacy of rhTM for human t-HUS, Honda and Kawasaki have reported that rhTM therapy might be of benefit to children with t-HUS [15, 16]. However, the number of patients in these reports was very small (n = 5), making it difficult to draw any conclusions from the two studies. Nevertheless, the results of our experimental study indicate that rhTM therapy may afford a useful treatment for t-HUS model mice in which inflammatory reactions and coagulation abnormalities occur concurrently due to treatment with LPS and Stx2. Hence, further studies on large populations of children with t-HUS are expected.

In conclusion, these results suggest that rhTM might be a useful treatment in the t-HUS mouse model via inhibition of further thrombin formation and amelioration of the hypercoagulant status as well as the suppression of inflammatory cytokines and chemokines.

ACKNOWLEDGEMENTS

The authors thank M. Tanji and K. Honzumi for assistance.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


Received for publication: 31.7.2014; Accepted in revised form: 24.12.2014

The efficacy of rhTM for HUS model mice