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Letter to the Editor Open Access

The Preventative Effects of Recombinant Thrombomodulin on Transplantation-Associated Coagulopathy after Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract

We investigated the preventive effects of recombinant thrombomodulin (rTM) on Transplantation Associated Coagulopathy (TAC) and TAC-related biomarkers in 271 patients after hematopoietic stem cell transplantation (HSCT). There were no significant differences between the rTM (+) or (-) groups and patient background, types of disease or HSCT regimens. When we examined patients with confirmed complications, all frequencies of aGVHD, VOD and/or TMA, as well as uncomplicated cases were significantly lower in the rTM (+) group. HMGB1 and MCP-1 showed a clear induction after transplantation, which peaked for HMGB1 at day 0 and for MCP-1 at day 7. Although MCP-1 levels did not exhibit significant differences between the two groups, HMGB1 levels in the rTM (+) group showed a significant reduction after day 4 compared with the rTM (-) group. The levels of PAI-1, sE-selectin and sVCAM-1 showed a significant increase in the groups that did not receive rTM. In contrast, the groups that received rTM did not show significant changes and significant differences were found between the two rTM-treated groups. Our multi-institutional study findings suggest that this agent is beneficial as part of preventive therapies for established TAC after HSCT.

Keywords: rTM; TAC; Stem cell transplantation; sE-selectin; HMGB1

Letter

Hematopoietic Stem Cell Transplantation (HSCT) involves specific serious transplant-related complications [1,2], and recovery from these complications is vital for achieving a successful HSCT outcome. Therefore, taking steps to mitigate coagulation- and Graft-Versus Host Disease (GVHD)-related complications following HSCT is very important. Several interactions between coagulation-related blood components and the fibrinolytic systems are involved in the progression of vascular angiopathy. Notably, plasminogen activator inhibitor (PAI)-1 plays an important role in the pathophysiology of many vascular abnormalities [3]. Recombinant thrombomodulin (rTM) is composed of the active extracellular domain of TM. Like membrane-bound TM, rTM binds to thrombin to inactive coagulation. The thrombin–rTM complex activates protein C to produce active protein C (APC), which in the presence of protein S inactivates factors VIIIa and Va, thereby inhibiting further thrombin formation [4]. Therefore, rTM might be useful for transplantation-associated coagulopathy (TAC) after HSCT. Indeed, there are some reports of the efficacies of therapies for TAC, such as veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA) [5,6]. However, the preventive effects of rTM for TAC or TAC-related biomarkers following HSCT are poorly understood. Here, we investigated the preventive effects of rTM for TAC and TAC-related biomarker levels after HSCT. To our knowledge, this report of a multi-institutional joint study is the first to document the potential ability of rTM to prevent TAC after HSCT.

The study cohort included 271 patients who underwent SCT between June 2011 and February 2014 at one of 24 institutions in Japan. All patients received allogeneic SCT (Table 1). The 161 male and 110 female allogeneic SCT patients ranged in age from 7 to 71 years (median: 45 years). Patient diagnoses consisted of 102 acute myeloid leukemia cases, 63 acute lymphoblastic leukemia cases, 38 myelodysplastic syndrome cases and 68 other diagnoses. Conditioning was applied as follows: total body irradiation for 175 patients and non-total body irradiation for 96 patients. The donor sources for transplantation were 139 bone marrow cells, 57 peripheral blood stem cells and 75 cord blood cells (Table 1). Written informed consent was obtained from all patients who were registered by faxing documents to Kansai Medical University prior to SCT. The rTM, consisting of daily doses of 380 units/kg (Asahi Kasei Pharma, Tokyo, Japan), was administered as a preventive therapy for TAC. This protocol was

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	rTM (-) (n=156)	rTM (+) (n=115)	P-value
Age, years, median (range)	44 (7-77)	47 (16-71)	0.3988
Sex, Male/Female	92/64	69/46	0.8651
Disease			
AML	61	41	0.6959
ALL	39	24	0.5290
MDS	21	17	0.7883
Other	35	33	0.3650
Conditioning regimen including TBI			
Yes	102	73	0.8823
No	54	42	-
Donor type			
Related	52	43	0.7493
Unrelated	104	72	-
Stem Cell Source			
Bone marrow	79	60	0.8875
Peripheral blood	39	18	0.1292
Umbilical cord blood	38	37	0.2868
Complication after HSCTC			
Known	113	96	0.4440
Yes, aGVHD	75	35	0.0149*
Yes, VOD and/or TMA	40	18	0.0424*
No	22	45	0.0025**
Unknown	43	19	0.0877
Survival at Day 60			
Known	108	89	0.5548
Yes	72	61	0.5136
No	36	24	0.7295
Unknown	48	22	0.0940
Quality of life			
Known	70	59	0.5336
Good	43	38	0.4756
Not so good	27	21	0.8652
Unknown	41	24	0.4177

rTM: Recombinant Thrombomodulin; AML: Acute Myeloid Leukemia; ALL: Acute Lymphocytic Leukemia; MDS: Myelodysplastic Syndrome; TBI: Total Body Irradiation; HSCT: Hematopoietic Stem Cell Transplantation; aGVHD: Acute Graft-versus-host Disease; VOD: Veno-occlusive Disease; TMA: Thrombotic Microangiopathy.
*: $P< 0.05$; **: $P< 0.01$

Table 1: Patient characteristics.

completed from days 4 to 14 after HSCT. An anticoagulation regimen of 5000 U heparin 24 h per day was used prior to rTM administration. Heparin or no anticoagulation therapy was also administered to the control groups.

Blood samples from patients were collected into tubes containing either sodium citrate or no anticoagulant; in the latter group, blood was allowed to clot at room temperature. Then, serum or citrated plasma was isolated by centrifugation for 20 min at 1000×g and 4°C. The serum was divided into aliquots and frozen at –30°C until use. As a positive control, recombinant proteins were used in each assay, as well as the standard solutions that were provided with the commercial kits. Interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein (MCP)-1, RANTES, sE-selectin and PAI-1 ELISA kits were purchased from BioSource International Inc (Camarillo, CA, USA). High mobility group box 1 (HMGB1) was measured using the HMGB1 ELISA Kit II (Shino-test Corp., Kanagawa, Japan). All ELISA kits were used according to the manufacturers' instructions. Data are expressed as the means ± SD and were analyzed by two-factor ANOVA for repeated measures as appropriate. Between-group comparisons were made using the Newman–Keuls and Scheffe's tests. Differences in characteristics between patient groups who used rTM or not were assessed using χ^2 tests of association for categorical values. All statistical analyses were performed using StatFlex (ver. 6) software and $P<0.05$ was used as a threshold for statistically significant differences.

There were no significant differences between the rTM (+) and (–) groups for patient background, type of disease or HSCT regimen (Table 1). When we examined patients who had confirmed complications, the frequencies of aGVHD and VOD and/or TMA were significantly lower in the rTM (+) groups (Table 1). In addition, the frequency of uncomplicated cases was significantly higher in the rTM (+) groups (Table 1). However, there were no significant differences with survival at day 60 and quality of life between two groups.

HMGB1 and MCP-1 levels clearly increased after transplantation, with HMGB1 peaking at day 0 and MCP-1 peaking at day 7 (Figure 1). Although MCP-1 levels did not show a significant difference between the two groups, HMGB1 levels in the rTM (+) group showed a significant reduction after day 4 compared with the rTM (–) group. Significant differences in IL-6, TNF-α and RANTES levels were not observed between the two groups. The levels of PAI-1, sE-selectin and sVCAM-1 showed a significant increase in the groups in which rTM was not used. In contrast, the groups in which rTM was used did not show significant changes, and significant differences were found between the two rTM-treated groups (Figure 1).

It is thought that the risk for TAC includes many factors, such as age of the patient, basal disease or the degree of disease remission before HSCT [7,8]. Additionally, previous reports have documented the effect of conditioning regimens that included TBI or not [9]. In our study, the increase of HMGB1 levels after HSCT was a very interesting finding, and suggested that HMGB1 plays an important role in the development of TAC following the HSCT conditioning treatment regimen [10]. Cutler et al. [11] reported that increased vascular endothelial cell dysfunction is a predictive indicator of VOD. Although the etiology of VOD remains unclear, we believe one of the causes of VOD is increased levels of proinflammatory cytokine, such as HMGB1 [12,13]. Therefore, the direct anti-inflammatory effect mediated via the rTM lectin domain is thought to play an important role in preventing VOD. Our findings suggest the possibility that rTM can help prevent TAC after allogeneic HSCT [10]. Recently, Ikezoe et al. [14] reported that rTM was useful to improve clinical outcomes of transplant recipients with coagulopathy. They exhibited that the treatment for coagulopathy by rTM significantly improved clinical ourcomes of patients at day 100 and dramatically prolonged patient's overall survival, while that was a single-institutional study.

We found that increased levels of PAI-1, sE-selectin and sVCAM-1 could be observed in the group not treated with rTM after HSCT. By contrast, levels of these molecules did not show significant changes in groups that received TM after HSCT. PAI-1 is synthesized in the liver and by endothelial cells, vascular smooth muscle cells and macrophages. PAI-1 expression can be regulated by many factors, including cytokines, oxidative stress and cellular signaling molecules. All patients with transplantation-related complications, especially patients with thrombotic complications, appear to have significant increases in the mean and maximum levels of PAI-1 during the observational period after HSCT [15,16]. A number of markers for endothelial injury and adhesion molecules are upregulated in patients with thrombotic complications in HSCT, including E-selectin, tissue

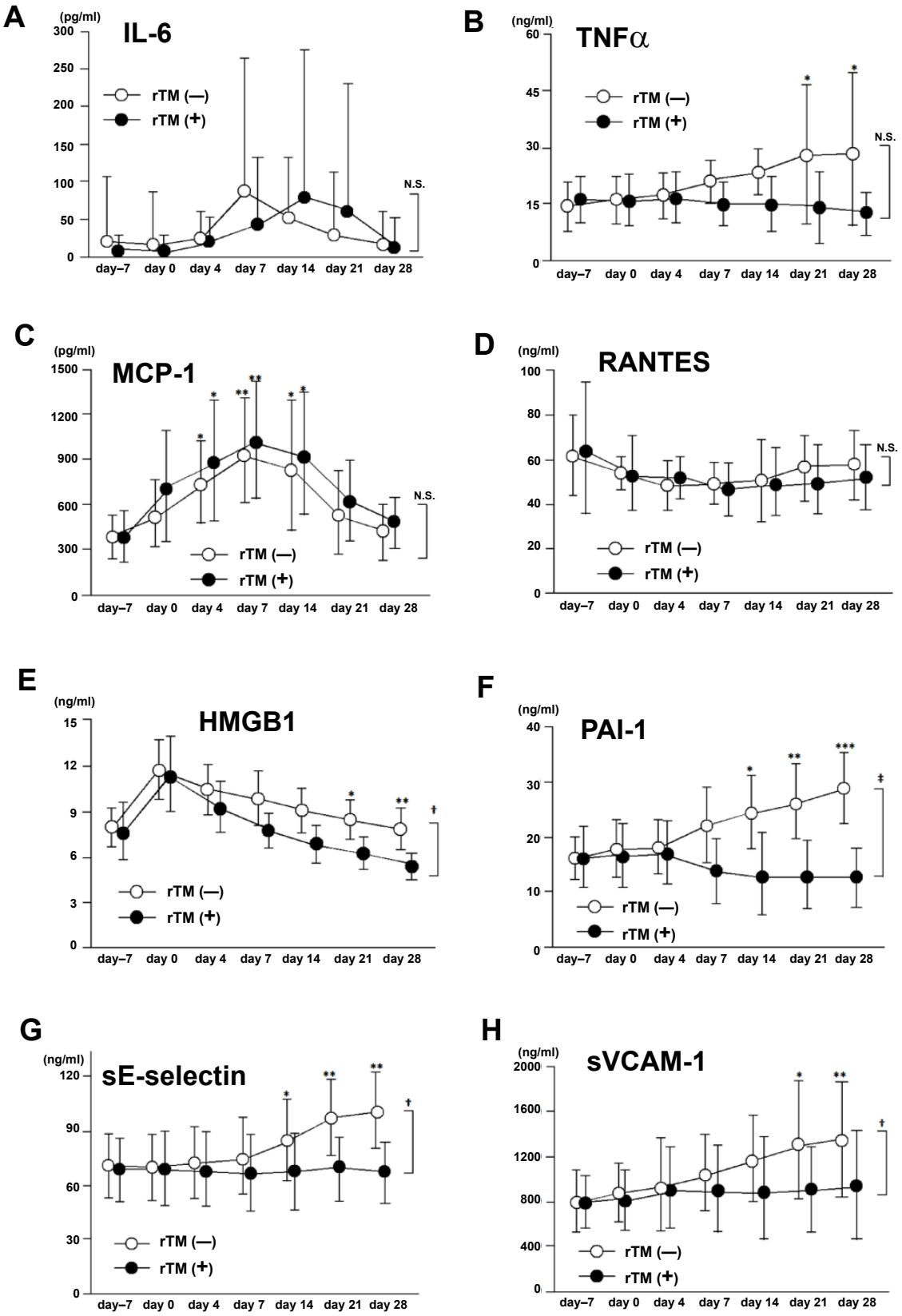

Figure 1: The levels of selected biomarkers before and after HSCT. Concentrations of (A) IL-6, (B) TNF-α, (C) MCP-1, (D) RANTES, (E) HMGB1, (F) PAI-1, (G) sE-selectin and (H) sVCAM-1 in patients with or without rTM treatment are shown. Data are shown as means ± SD. For comparisons versus day 0: *P<0.05; **P<0.01; ***P<0.001. For ANOVA analysis between two groups with or without rTM treatment: †P<0.05; ‡P<0.01; NS: Not Significant.

factor and PAI-1 [11-13,15-17]. Our data presented here also support these previous reports.

In conclusion, rTM could significantly reduce HMGB1 and PAI-1 levels after HSCT. Additionally, patients who received preventive rTM exhibited significantly lower frequencies of VOD and/or TMA, as well as aGVHD compared to patients who did not receive rTM. Therefore, the results of our multi-institutional study suggest that trTM is beneficial when used as a preventive therapy for established TAC after HSCT.

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