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ORIGINAL ARTICLE

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Effects of thrombomodulin alfa on hemostatic parameters in disseminated intravascular coagulation: Post hoc analysis of a phase 3 randomized controlled trial

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Abstract

Background: The efficacy and safety of thrombomodulin alfa (TM-α), a cofactor protein promoting thrombin-mediated protein C activation, have been examined in a phase 3 randomized, double-blinded, parallel-group trial in Japan. We have previously reported that TM-α is noninferior to heparin for the resolution of disseminated intravascular coagulation (DIC).

Objective: To investigate the basis for the efficacy of TM-α in the phase 3 clinical trial in Japan through post hoc analysis of coagulation and fibrinolysis parameters.

Patients/Methods: The 227 patients of the full analysis set population described in the original phase 3 trial in Japan were included in this analysis. Changes in parameters between before and after TM-α or heparin administration in each of the two patient groups, with underlying diseases of either hematologic malignancy or infection, were studied separately and results were compared between TM-α and heparin treatment groups in a post hoc manner.

Results: TM-α administration did not prolong activated partial thromboplastin time but significantly decreased thrombin-antithrombin complex levels compared with heparin treatment. TM-α administration reduced consumption of endogenous anticoagulants such as antithrombin and protein C by DIC, compared with the heparin group. DIC scores were decreased in both TM-α and heparin groups during the 6-day treatment.

Conclusion: TM-α can alleviate intravascular coagulation and consumption of anticoagulants without extending coagulation times. This may be associated with the relatively low risk of bleeding during TM-α treatment.

KEYWORDS

disseminated intravascular coagulation, heparin, protein C, thrombin, thrombomodulin

Essentials

- Mechanisms of action of thrombomodulin alfa (TM-α) are not yet clinically confirmed in patients.
- We evaluated hemostatic parameters in patients with disseminated intravascular coagulation.
- TM-α inhibited thrombin generation without extending clotting times in patients.
- Activated protein C–dependent mechanisms may play key roles in the clinical efficacy of TM-α.

1 | INTRODUCTION

Disseminated intravascular coagulation (DIC) is an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. DIC can originate from and cause damage to the microvasculature, which can produce organ dysfunction if sufficiently severe.[1]. DIC frequently complicates hematologic malignancy, infection, and solid tumor.[2-4] In recent years, some mechanisms involved in the pathogenesis of DIC have been clarified, such as tissue factor triggering thrombin generation, platelet activation, microparticle release, downregulation of natural anticoagulants, inhibition of fibrinolysis, formation of neutrophil extracellular traps, crosstalk coagulation, and inflammatory pathways.[5]

The management of DIC includes treatment of underlying diseases, supplementation of depleted coagulation and fibrinolysis factors and associated regulatory factors, and inhibition of aberrant activation of coagulation.[2,6,7] Thrombomodulin, an anticoagulant glycoprotein expressed on the surface of vascular endothelial cells, is an attractive therapeutic target for regulating aberrant activation of coagulation.[8] Thrombomodulin binds to thrombin to inactivate coagulation, and the thrombin-thrombomodulin complex 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.[8] The thrombomodulin–protein C system thus converts thrombin from a procoagulant enzyme to an anticoagulant enzyme on vascular endothelial cells, which prevents intravascular coagulation, inflammation, and endothelial barrier disruption. Reinforcement of this system could be beneficial in the management of DIC.[9]

Thrombomodulin alfa (TM-α) is a recombinant human soluble thrombomodulin composed of the active, extracellular domain of thrombomodulin.[10-12] In a phase 3 clinical trial in Japan, TM-α was noninferior to heparin in terms of DIC resolution in patients with DIC associated with infection or hematologic malignancy.[13,14] Recent meta-analyses suggested that TM-α might reduce 28-day mortality in patients with sepsis-associated coagulopathy.[15,16]

The mechanism of action differs between heparin and TM-α.[11,12] Heparin binds to antithrombin, accelerating antithrombin-dependent inhibition of clotting enzymes, particularly thrombin and factor Xa. Meanwhile, TM-α binds to thrombin, directly inhibiting the procoagulant activity of thrombin, or downregulating thrombin generation through the activation of protein C. Previous preclinical studies have suggested that the latter mechanism of action may be predominant at clinical concentrations of TM-α,[11,12] but this hypothesis has not actually been clinically demonstrated in DIC patients. The purpose of this study was to gain insights into the mechanism of action for TM-α in comparison with heparin through analyzing clinical data.

2 | METHODS

2.1 | Patients and interventions

This post hoc analysis used data from the phase 3 TM-α clinical study reported by Saito et al.[13] This study was a double-blinded, randomized, parallel-group clinical study comparing TM-α to heparin among patients with DIC for whom the primary underlying pathology was either hematologic malignancy or infection. DIC was diagnosed according to the criteria proposed by the Japanese Ministry of Health and Welfare (JMHW).[17] Patients with potentially fatal or life-threatening bleeding or patients at high risk of such a condition were excluded. Other inclusion and exclusion criteria are described in the Appendix S1 and two previous reports.[13,14] The phase 3 study was conducted in compliance with good clinical practice and the ethical principles of the Declaration of Helsinki. Prior approval was obtained from the ethics review boards of all participating institutions. Written informed consent was obtained from all patients or acceptable representatives.[13] Details of the study are reported in the two previous reports.[13,14]

In total, 234 patients consented to participate, and study drugs were eventually administered to a total of 232 patients. Of these, 5 patients were excluded from the full analysis set (FAS) because of double registration (n = 1), insufficient DIC score (n = 1), or inappropriate underlying disease (n = 3). In the present study, analyses were carried out on 227 FAS patients (TM-α, n = 114; heparin, n = 113). Either TM-α (0.06 mg/kg infused over a period of 30 minutes, once daily) or heparin (8 U/kg/h for 24 hours) was intravenously administered for 6 consecutive days (Appendix S1). During the period of study drug treatment, concurrent use of drugs that could influence the efficacy assessment of TM-α (including anticoagulants, antithrombin agents, antiplatelet agents, and fibrinolytic agents) was prohibited. However, concurrent use of blood products (fresh frozen plasma and platelet concentrates) and heparin given with the objective of preventing clotting at the site of catheterization (≤1000 U/day) was permitted. No differences were seen in the usage of these agents between groups. Blood samples were collected before study drug administration and at 7 days after the start of study drug administration or at discontinuation (Appendix S1).

2.2 | Evaluation of patients

The prospectively defined primary efficacy end point was the DIC resolution rate (rate of recovery from DIC) as assessed at 7 days after the start of infusion (or withdrawal) using JMHW DIC criteria. In this post hoc study, we also assessed DIC resolution rates using DIC scores as proposed by the ISTH. DIC scores were determined before and after study drug infusion, and DIC resolution rate was assessed in patients who met ISTH DIC criteria before study drug infusion. Resolved DIC status (no DIC) was defined as an ISTH DIC score < 5 points. For elevated fibrin-related markers, D-dimer was used (<1 μg/mL, 0 points; ≥ 1 μg/mL but < 10 μg/mL, 2 points; ≥10 μg/mL, 3 points). For prolonged prothrombin time (PT), PT ratio was used (<1.25, 0 points; ≥1.25 but < 1.67, 1 point; ≥1.67, 2 points).

Second, we evaluated changes in hemostatic parameters from before to after administration of TM-α or heparin as the rate or amount of change. As for hemostatic parameters, we evaluated fibrin and fibrinogen degradation products (FDP), platelet count, fibrinogen, PT ratio, and activated partial thromboplastin time (APTT), thrombin-antithrombin complex (TAT), plasmin-plasmin inhibitor complex (PIC), D-dimer, α_2 plasmin inhibitor (α_2PI), protein C, plasminogen activator inhibitor-1 (PAI-1), fibrin monomer complex (FMC), and antithrombin. The rate of change was defined as the value at the end of study drug infusion (or withdrawal) minus the value at baseline divided by the value at baseline. The amount of change was defined as the value at the end of study drug infusion (or withdrawal) minus the value at baseline.

Finally, we conducted a subgroup analysis according to baseline levels of protein C and antithrombin, important agents of TM-α and heparin treatment, respectively, and evaluated DIC resolution rates. In this analysis, we used the preplanned DIC resolution rate as assessed using JMHW DIC criteria.

With regard to the usage of antithrombin products after the completion of administration, the number and percentage of patients who used antithrombin products from the completion of administration or discontinuation to day 14 after the start of administration were calculated in relation to primary underlying diseases for DIC and drugs.

2.3 | Laboratory methods

TAT, PIC, D-dimer, α_2PI, protein C, PAI-1, FMC, and antithrombin were measured by a central testing facility (SRL Medisearch Inc). After blood sample collection, plasma samples were immediately prepared and stored frozen according to "procedures for collection of samples" in the protocol. Frozen samples were submitted to and analyzed at SRL Medisearch Inc according to the verified manuals attached to measurement kits. TAT was measured by an enzyme immunoassay method (thrombin/antithrombin III complex TAT "S" kit; SRL Medisearch Inc, Tokyo, Japan). PIC was measured using a latex photometric immunoassay (LPIA) method (LPIA ACE PPI II Kit; Mitsubishi Kagaku Iatron, Tokyo, Japan), as was PAI-1 (LPIA/tPAI test; Mitsubishi Kagaku Iatron). D-dimer was measured by a latex immunoturbidimetric method (Cobas Reagent D-dimer Kit; Roche Diagnostics, Rotkreuz, Switzerland), as was FMC (AutoLIA FM; Nissui Seiyaku, Tokyo, Japan). Antithrombin (Testzyme S ATIII; Chromogenix, Uppsala, Sweden) and α_2PI (Testzyme S APL; Chromogenix) were measured by a chromogenic synthetic substrate method. Protein C (STA Reagent Series, Protein C (clot); Diagnostica Stago, Parsippany, NJ) was measured based on APTT clotting time.

FDP, fibrinogen, platelet count, PT ratio, and APTT were measured at each hospital immediately. Precision management of laboratory data was managed by the Prefectural Association of Medical Technologists.

2.4 | Statistical analysis

For this post hoc analysis, patients were classified into two groups based on the underlying pathology (hematologic malignancy or infection). For analysis of the changes in platelet count or JMHW DIC score, patients were classified into leukemia and nonleukemia groups based on JMHW DIC criteria (Appendix S1). DIC resolution rate was calculated for each underlying disease, and the absence of qualitative interaction between underlying disease and drug was confirmed using descriptive analysis and the Breslow-Day test. Changes in

TABLE 1 Baseline hemostatic parameters

	Infection			
	TM-α group		Heparin group	
	n	Median (Q1, Q3)	n	Median (Q1, Q3)
FDP (μg/mL)	50	30.9 (16.0, 56.7)	52	30.0 (21.7, 57.2)
Fibrinogen (mg/dL)	50	370 (283, 515)	52	397 (220, 523)
PT ratio	50	1.14 (1.04, 1.29)	52	1.16 (1.10, 1.30)
APTT (s)	50	37.6 (32.7, 46.2)	49	39.8 (36.8, 46.2)
TAT (ng/mL)	50	17.1 (9.1, 30.3)	52	21.1 (11.4, 39.0)
PIC (μg/mL)	50	2.3 (1.2, 5.0)	52	1.9 (1.2, 3.7)
D-dimer (μg/mL)	50	22.4 (10.1, 46.8)	52	29.0 (12.4, 46.5)
α_2PI (%)	50	80 (70, 91)	51	79 (65, 97)
protein C (%)	50	35 (26, 47)	52	33 (24, 45)
PAI-1 (ng/mL)	50	39 (23, 108)	51	56 (31, 96)
FMC (μg/mL)	50	40.1 (10.0, 119.0)	51	21.0 (8.3, 75.3)
Antithrombin (%)	50	60 (51, 75)	52	62 (47, 79)
PLT ($\times 10^9$/L)[a]	46	40 (24, 52)	45	47 (32, 68)

Abbreviations: α_2PI, α_2 plasmin inhibitor; APTT, activated partial thromboplastin time; FDP, fibrin and fibrinogen degradation products; FMC, fibrin monomer complex; PAI-1, plasminogen activator inhibitor-1; PIC, plasmin-plasmin inhibitor complex; PLT, platelet count; PT, prothrombin time; Q1, first quartile; Q3, third quartile.; TAT, thrombin-antithrombin complex; TM-α, thrombomodulin alfa.

[a]Data from the nonleukemia group, diagnosed according to DIC criteria established by the Japanese Ministry of Health and Welfare (Appendix S1).

hemostatic parameters from before to after administration of TM-α or heparin were studied separately in each patient group and the results were compared between TM-α and heparin groups. Median and two-tailed 95% confidence interval (CI) were calculated for differences in the amount or rate of changes from start of drug administration for each hemostatic parameter. Analysis was also applied to intergroup differences. If the 95% CI did not cross zero, the difference was judged as statistically significant.

TABLE 2 DIC resolution rate as assessed using ISTH DIC and JMHW DIC diagnostic criteria

	Infection	
	DIC resolution rate, n (%)	Point estimate of difference (95% CI)
ISTH criteria		
TM-α group	24/33 (72.7)	9.4 (−13.6 to 32.4)
Heparin group	19/30 (63.3)	
JMHW criteria[a]		
TM-α group	32/48 (66.7)	11.8 (−7.3 to 30.9)
Heparin group	28/51 (54.9)	

Abbreviations: CI, confidence interval;DIC, disseminated intravascular coagulation; JMHW, Japanese Ministry of Health and Welfare; TM-α, thrombomodulin alfa.

[a]Description from Saito H, et al.[13]

3 | RESULTS

3.1 | Baseline hemostatic parameters

Baseline characteristics of the patients have been reported previously.[13] No marked differences existed in baseline hemostatic parameters between the TM-α and heparin groups (Table 1 and Table S1).

3.2 | DIC resolution as assessed by JMHW criteria and ISTH criteria

DIC resolution rate was assessed using both JMHW and ISTH criteria (Table 2 and Table S2). The prospectively defined primary efficacy end point, DIC resolution rate as assessed by JMHW criteria, was assessed in 224 of the 227 FAS population, excluding 3 patients for whom DIC scores were not evaluated. DIC resolution rate was assessed in 147 patients (infection, n = 63; hematologic malignancy, n = 84) who met the ISTH DIC criteria. DIC resolution rates for TM-α and heparin groups were 72.7% and 63.3%, respectively, in patients with DIC associated

TABLE 3 Amount or rate of change in hemostatic parameters from before to after administration and intergroup differences between TM-α and heparin groups

	Infection		
	Amount or rate of change from before to after administration		
	TM − α group Median (Q1, Q3)	Heparin group Median (Q1, Q3)	Intergroup difference between TM-α and heparin groups (95% CI)
FDP (rate of change, %)	−57.9 (−77.6, −32.5)	−51.5 (−68.9, −19.4)	−9.0 (−23.9 to 4.1)
Fibrinogen (amount of change, mg/dL)	−32.0 (−148.0, 39.0)	−21.0 (−94.0, 35.0)	−7.5 (−59.2 to 47)
PT ratio (amount of change)	0.02 (−0.06, 0.09)	−0.02 (−0.08, 0.11)	0.03 (−0.04 to 0.08)
APTT (amount of change, s)	0.1 (−7.9, 5.4)	4.2 (−2.4, 12.6)	−4.8* (−9.7 to − 0.4)
TAT (rate of change, %)	−53.0 (−74.2, −7.3)	−45.3 (−63.9, −3.6)	−9.1* (−26.5 to − 8.3)
PIC (rate of change, %)	−34.0 (−64.0, 10.0)	−25.0 (−50.0, 6.3)	−6.5 (−27.6 to 15.3)
D − dimer (rate of change, %)	−59.8 (−78.3, −31.3)	−52.3 (−79.0, −20.2)	−6.2 (−21.5 to 7)
α_2PI (amount of change, %)	−1 (−8, 19)	−1 (−16, 7)	5 (−2 to 13)
Protein C (amount of change, %)	8 (0, 33)	8 (0, 24)	1 (−6 to 9)
PAI − 1 (rate of change, %)	3 (−32, 53)	5 (−35, 150)	−11 (−50 to 22)
FMC (rate of change, %)	−59.5 (−87.1, −12.0)	−36 (−87.8, 4.9)	−8.0 (−43.5 to 5.2)
Antithrombin (amount of change, %)	9 (−9, 18)	−7 (−16, 7)	14* (6 to 23)
PLT (amount of change, $\times 10^9$/L)[a]	59 (33, 185)	63 (1, 121)	25 (−11 to 62)

Note: Median of difference between TM-α and heparin groups is based on Mann-Whitney-Wilcoxon statistics. * 95% confidence interval (CI) does not include zero.

Abbreviations: α_2PI, α_2 plasmin inhibitor; APTT, activated partial thromboplastin time; FDP, fibrin and fibrinogen degradation products; FMC, fibrin monomer complex; PAI-1, plasminogen activator inhibitor-1; PIC, plasmin-plasmin inhibitor complex; PLT, platelet count; PT, prothrombin time; Q1, first quartile; Q3, third quartile.; TAT, thrombin-antithrombin complex; TM-α, thrombomodulin alfa.

[a]Data from the nonleukemia group diagnosed according to disseminated intravascular coagulation criteria established by the Japanese Ministry of Health and Welfare (Appendix S1).

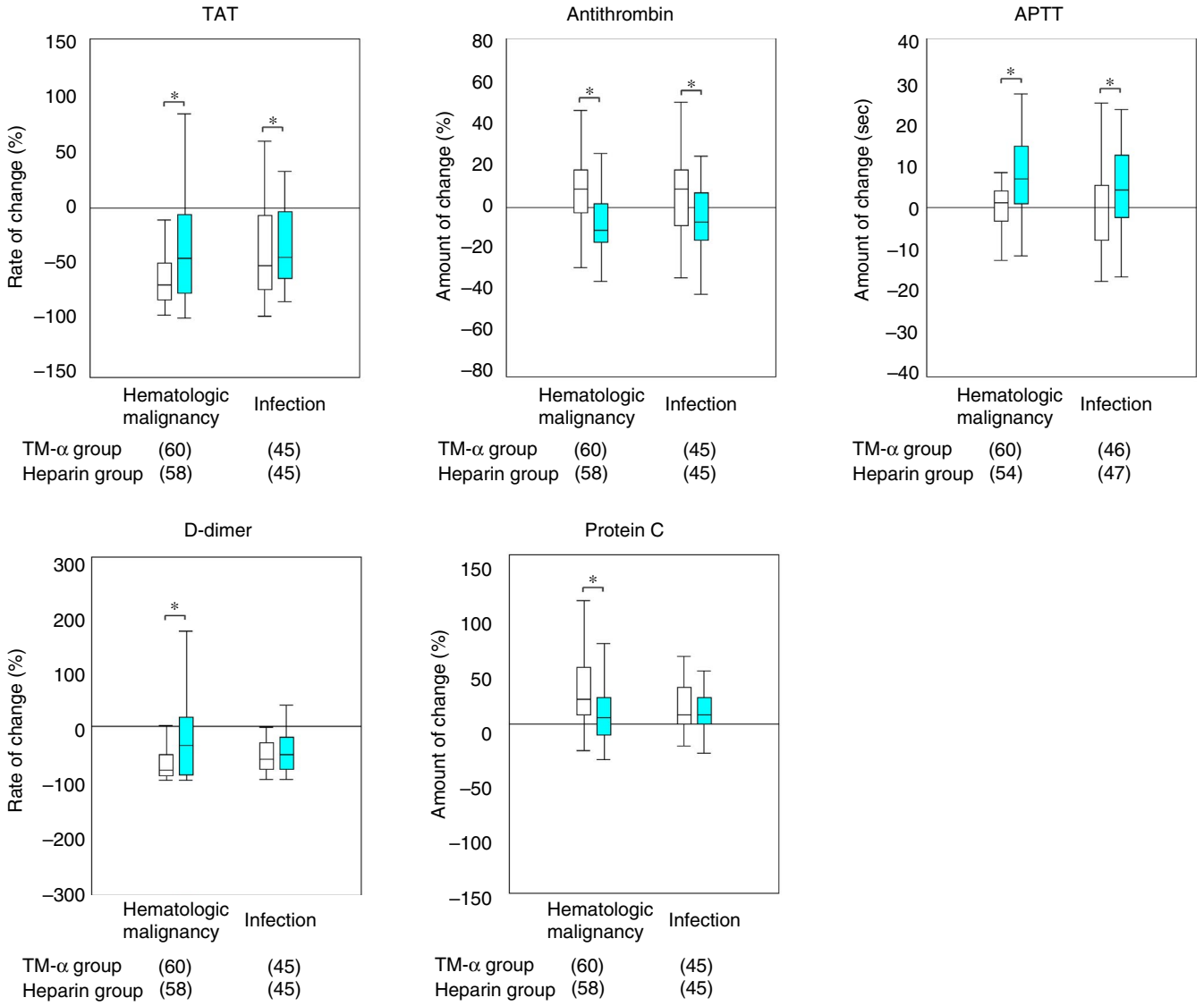

FIGURE 1 Changes in hemostatic parameters from before to after administration of TM-α and heparin. Changes in thrombin-antithrombin complex (TAT), antithrombin, activated partial thromboplastin time (APTT), D-dimer, and protein C levels are shown. Data are shown as box plots with lower extreme, lower quartile, median, upper quartile, and upper extreme values. White boxes represent changes in the TM-α group and blue boxes represent changes in the heparin group. Numbers of patients in each group category are also shown. Rectangles represent lower and upper limits of the interquartile range, and median values are demarcated inside rectangles. Vertical lines ("whiskers") represent the spread of data. Upper line represents the upper, or third, quartile plus 1.5 × (interquartile range), and lower line represents the lower, or first, quartile minus 1.5 × (interquartile range). Outliers are not indicated. Rate of change: value at end of study drug infusion (or withdrawal) minus value at baseline divided by value at baseline. Amount of change: value at end of study drug infusion (or withdrawal) minus value at baseline. Median difference between groups is based on Mann-Whitney-Wilcoxon statistics. *Difference judged as statistically significant

with infection (difference 9.4%; 95% CI −13.6-32.4; Table 2), and were 69.0% and 26.2%, respectively, in patients with hematologic malignancy (difference, 42.9%; 95% CI, 23.6-62.2; Table S2).

3.3 | Changes in hemostatic parameters

Most parameters reflecting coagulation and fibrinolysis abnormalities, such as FDP, TAT, PIC, and D-dimer, decreased after administration in both drug groups regardless of underlying disease (Table 3 and Table S3). Compared with heparin administration, TM-α administration resulted in a greater decrease in TAT and a greater increase in antithrombin values for both underlying diseases (Figure 1, Table 3, and Table S3). Nevertheless, prolongation of APTT was significantly lower in the TM-α group than in the heparin group for both underlying diseases (Figure 1, Table 3, and Table S3). In patients with hematologic malignancy, TM-α administration resulted in a greater decrease in D-dimer value and a greater increase in protein C when compared with heparin administration (Figure 1, Table S3).

TABLE 4 DIC resolution rates in relation to baseline antithrombin and protein C levels

	Infection				
	n	TM-α group number of patients (%)		Heparin group number of patients (%)	
Baseline antithrombin (%)					
<50	25	9/11	(81.8)	7/14	(50.0)
≥50 to < 70	39	13/20	(65.0)	12/19	(63.2)
≥70	35	10/17	(58.8)	9/18	(50.0)
Baseline protein C (%)					
<20	15	3/7	(42.9)	3/8	(37.5)
≥20 to < 50	64	20/31	(64.5)	19/33	(57.6)
≥50	20	9/10	(90.0)	6/10	(60.0)

Abbreviation: TM-α, thrombomodulin alfa.

3.4 | Resolution of DIC in relation to baseline antithrombin and protein C levels

Baseline antithrombin levels were ≥ 70% in 102 of 125 patients (82%) with hematologic malignancies (Table S4), and were < 70% in 64 of 99 patients (65%) with infection (Table 4). In patients with infection, the DIC resolution rate for the TM-α group was high irrespective of baseline antithrombin level. In the heparin group, the DIC resolution rate in patients with baseline antithrombin < 50% was not noticeably different from that in patients with baseline antithrombin ≥ 50 to < 70% or ≥ 70% (Table 4). DIC resolution rate was relatively low in patients with baseline protein C < 20% in both treatment groups, although the number of cases was small (Table 4).

3.5 | Usage of antithrombin products after completion of study drug administration

In both underlying diseases, antithrombin levels decreased after administration of heparin, whereas antithrombin levels increased after administration of TM-α (Figure 1). Although concurrent use of anticoagulants, including antithrombin products, was prohibited during the drug administration period, usage of antithrombin products after completion of study drug administration was permitted and subject to investigation. The percentages of patients who used antithrombin products after completion of TM-α and heparin administration were 10.0% (5/50) and 19.2% (10/52), respectively, in patients with DIC associated with infection, and 3.1% (2/64) and 16.4% (10/61), respectively, in patients with hematologic malignancy. For both underlying diseases, the percentage of patients who used antithrombin products after completion of study drug administration was higher for the heparin group than for the TM-α group, probably reflecting poor recovery of antithrombin levels at the end of study drug administration in the heparin group.

3.6 | Hemostatic parameters during administration of TM-α and heparin

Actual test values in the present study are shown in Table S5 and Figures S1-S3. Prolongation of APTT was recognized only in the heparin group for both underlying diseases (Figure S1). JMHW DIC scores gradually decreased during the 6-day treatment in both TM-α and heparin groups for both underlying diseases (Figure S2). Changes in hemostatic parameters from baseline are shown as spaghetti plots in Figure S3.

4 | DISCUSSION

In this post hoc analysis of a phase 3 study, we confirmed that TM-α significantly decreased TAT levels without extending APTT in DIC patients when compared with heparin. Considering that direct thrombin inhibitors, such as argatroban and hirudin, extend APTT, it appears reasonable to consider that TM-α might not directly inhibit the procoagulant activity of thrombin, but instead inhibits thrombin generation through activation of protein C in clinical settings.

The efficacy of recombinant APC (rAPC) was reported in patients with severe sepsis[18] or overt DIC.[19] Although rAPC and TM-α share the same mechanism of anticoagulation, TM-α may offer some advantages over rAPC. In contrast to the delivery of premade APC to sites of microthrombosis in defiance of plasma serine protease inhibitors, TM-α–mediated APC generation on-site can minimize redundant APC in the systemic circulation.[20] In fact, plasma level of free APC was not increased with TM-α treatment and remained at about 0-10 ng/mL throughout TM-α treatment,[20] and APTT was not prolonged in this setting (Figure 1), while plasma level of APC during rAPC treatment was about 45 ng/mL[21] and APTT was prolonged.[22] This may be associated with the relatively low risk of bleeding during TM-α treatment.[23] Nevertheless, anticoagulant effects of TM-α as evidenced by the decrease in TAT levels (Figure 1) were as good as, if not better than, those with rAPC.[12,13,22] TM-α thus does not increase plasma APC, instead increasing APC only at sites of microthrombosis (Figure 2). TM-α is thought to act locally at sites of microthrombosis, rather than systemically.

The contribution of plasma antithrombin levels to the therapeutic effects of heparin represents an interesting subject of investigation. Plasma antithrombin levels can be decreased in patients with infection, and decreased antithrombin levels are associated with poor outcome.[24-26] However, contrary to expectations, in the heparin group, the DIC resolution rate among patients with baseline antithrombin < 50% was not noticeably different from that among patients with baseline antithrombin ≥ 50 to < 70% or ≥ 70% (Table 4). By contrast, the DIC resolution rate in the TM-α group was relatively high among patients with baseline antithrombin < 50% compared to those with baseline antithrombin ≥ 50% (Table 4). One reason for this may be that TM-α–mediated APC generation requires thrombin, which can be irreversibly inactivated by antithrombin. Lower levels of antithrombin may thus be rather favorable for thrombin-TM-α–mediated APC generation.[20] Furthermore,

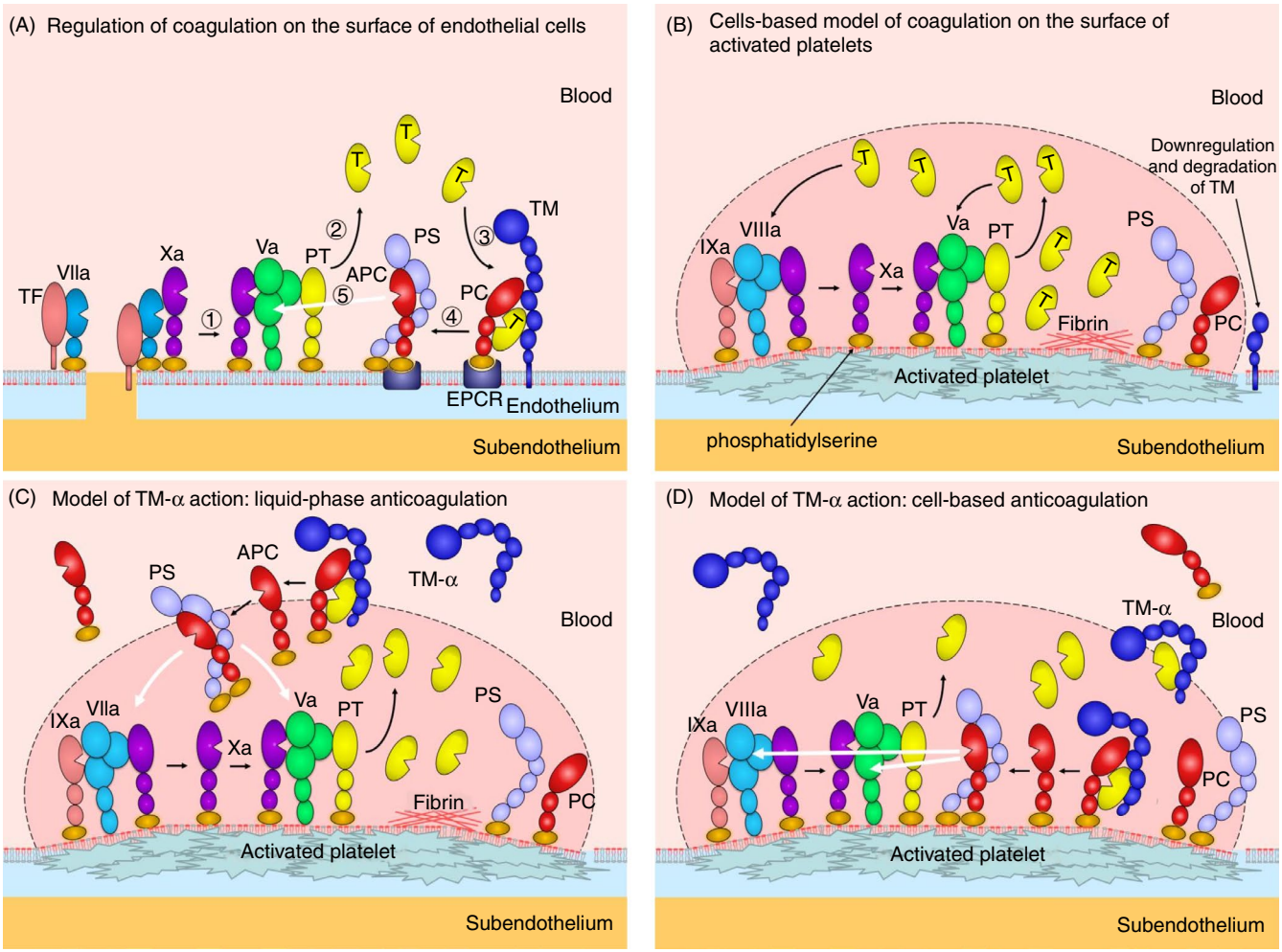

FIGURE 2 Schematic overview of a cell-based model of anticoagulation by TM-α. (A) On the surface of endothelial cells, thrombomodulin (TM) and endothelial cell protein C receptor (EPCR) coordinately promote thrombin-mediated activation of protein C (PC). Activated protein (APC) then inactivates activated factor V (Va) in the presence of protein S, thereby inhibiting further thrombin formation. (B) In the cell-based model of coagulation,[34] coagulation is amplified and propagated on the platelet surface. Gamma-carboxyglutamic acid (Gla)-domain-containing coagulation factors (VII, IX, X, prothrombin) preferentially binds to phosphatidylserine clusters on the plasma membrane of activated cells, such as platelets, to efficiently generate large amounts of thrombin and form fibrin-rich microthrombi. At this time, Gla-domain-containing anticoagulation factors (protein C, protein S) also accumulate at the same membrane surface in microthrombi. (C, D) TM-α binds thrombin around microthrombi and activates protein C, similar to endothelial TM. It is unknown whether TM-α may act in the liquid-phase (C) or on the surface of activated cells (D). We propose here that TM-α may act on the surface of activated cells at sites of microthrombosis. This hypothesis is based on the following findings: (1) TM-α treatment did not increase plasma levels of free APC[20]; (2) TM-α treatment did not prolong APTT (Figure 1); (3) TM-α treatment efficiently decreased plasma levels of TAT (Figure 1). Thus, the TM-α-APC system is thought to act locally on the surface of activated cells, rather than act in the systemic circulation. VII, factor VII; IX, factor IX; X, factor X; TAT, thrombin-antithrombin complex; APTT, activated partial thromboplastin time; TF, tissue factor; VIIa, activated factor VII; Xa, activated factor X; Va, activated factor V; PT, prothrombin; T, thrombin; TM, thrombomodulin; PC, protein C; EPCR, endothelial protein C receptor; PS, protein S; APC, activated protein C; IXa, activated factor IX; VIIIa, activated factor VIII; TM-α, thrombomodulin alfa

plasma antithrombin levels were increased after administration of TM-α, but were decreased after administration of heparin (Table 3 and Table S3). Consumption of antithrombin may be associated with decreased antithrombin levels after heparin administration, and resolution of DIC by TM-α administration may lead to the recovery of antithrombin synthesis in the liver and the retention of antithrombin within the vasculature. Furthermore, the usage of antithrombin products after the completion of study drug administration in the TM-α group was lower when compared with the heparin group, suggesting resource-saving effects of TM-α.

The contribution of protein C levels to the therapeutic effects of TM-α is another subject of investigation. PC is a plasma protein required for TM-α to fully express its anticoagulant activity. Plasma protein C levels can be decreased in patients with infection and decreased protein C levels are associated with poor outcome.[25,26] In patients with baseline protein C < 20% in the TM-α group, the DIC resolution rate was low (Table 4 and Table S4). DIC was not resolved in any of the four patients with baseline protein C < 10% in the TM-α group (data not shown). The contribution of protein C levels to the therapeutic effects of TM-α should be investigated in a greater number of patients in the future.

Crosstalk between inflammation and coagulation has been implicated in the pathogenesis of DIC.[26,27] Thrombomodulin not only regulates blood coagulation, but also suppresses inflammation via its lectin-like domain[28-32] and via APC.[33] Determination of whether and how anti-inflammatory activity is involved in the efficacy of TM-α will be important.

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RELATIONSHIP DISCLOSURE

TI and IM received research grants and speaking honoraria from Asahi Kasei Pharma Corporation. NA received speaking honoraria from Asahi Kasei Pharma Corporation. GH is an employee of Asahi Kasei Pharma Corporation. The other authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

TI, IM, SS, YY, NA, AH, GH, and HS all participated in the study design and interpretation of data. GH provided data. TI and IM prepared a draft of the manuscript. SS, YY, NA, AH, GH, and HS critically reviewed multiple drafts of the manuscript.

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REFERENCES

1. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001;86:1327–30.
2. Gando S, Levi M, Toh C-H. Disseminated intravascular coagulation. Nat Rev Dis Primers. 2016;2:16037.
3. Thachil J, Falanga A, Levi M, Liebman H. Di Nisio M; Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis. Management of cancer-associated disseminated intravascular coagulation: guidance from the SSC of the ISTH. J Thromb Haemost. 2015;13:671–5.
4. Levi M. Clinical characteristics of disseminated intravascular coagulation in patients with solid and hematological cancers. Thromb Res. 2018;164(Suppl 1):S77–S81.
5. Papageorgiou C, Jourdi G, Adjambri E, Walborn A, Patel P, Fareed J, et al. Disseminated intravascular coagulation: an update on pathogenesis, diagnosis, and therapeutic strategies. Clin Appl Thromb Hemost. 2018;24(9_suppl):8S–28S.
6. Wada H, Wakita Y, Nakase T, Shimura M, Hiyoyama K, Nagaya S, et al. Outcome of disseminated intravascular coagulation in relation to the score when treatment was begun. Mie DIC Study Group. Thromb Haemost. 1995;74:848–52.
7. Wada H, Asakura H, Okamoto K, Iba T, Uchiyama T, Kawasugi K, et al. Expert consensus for the treatment of disseminated intravascular coagulation in Japan. Thromb Res. 2010;125:6–11.
8. Esmon NL, Owen WG, Esmon CT. Isolation of a membrane-bound cofactor for thrombin-catalyzed activation of protein C. J Biol Chem. 1982;257:859–64.
9. Maruyama I. Recombinant thrombomodulin and activated protein C in the treatment of disseminated intravascular coagulation. Thromb Haemost. 1999;82:718–21.
10. Suzuki K, Kusumoto H, Deyashiki Y, Nishioka J, Maruyama I, Zushi M, et al. Structure and expression of human thrombomodulin, a thrombin receptor on endothelium acting as a cofactor for protein C activation. EMBO J. 1987;6:1891–7.
11. Mohri M, Gonda Y, Oka M, Aoki Y, Gomi K, Kiyota T, et al. The antithrombotic effects of recombinant human soluble thrombomodulin (rhsTM) on tissue factor-induced disseminated intravascular coagulation in crab-eating monkeys (*Macaca fascicularis*). Blood Coagul Fibrinolysis. 1997;8:274–83.
12. Mohri M, Sugimoto E, Sata M, Asano T. The inhibitory effect of recombinant human soluble thrombomodulin on initiation and extension of coagulation–a comparison with other anticoagulants. Thromb Haemost. 1999;82:1687–93.
13. Saito H, Maruyama I, Shimazaki S, Yamamoto Y, Aikawa N, Ohno R, et al. Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: results of a phase III, randomized, double-blind clinical trial. J Thromb Haemost. 2007;5:31–41.
14. Aikawa N, Shimazaki S, Yamamoto Y, Saito H, Maruyama I, Ohno R, et al. Thrombomodulin alfa in the treatment of infectious patients complicated by disseminated intravascular coagulation: subanalysis from the phase 3 trial. Shock. 2011;35:349–54.
15. Valeriani E, Squizzato A, Gallo A, Porreca E, Vincent JL, Iba T, et al. Efficacy and safety of recombinant human soluble thrombomodulin in patients with sepsis-associated coagulopathy: a systematic review and meta-analysis. J Thromb Haemost. 2020;18(7):1618–25.
16. Yamakawa K, Levy JH, Iba T. Recombinant human soluble thrombomodulin in patients with sepsis-associated coagulopathy (SCARLET): an updated meta-analysis. Crit Care. 2019;23:302.
17. Kobayashi N, Maekawa T, Takada M, Tanaka H, Gonmori H. Criteria for diagnosis of DIC based on the analysis of clinical and laboratory findings in 345 DIC patients collected by the Research Committee on DIC in Japan. Bibliotheca Haematologica. 1983;49:265–75.
18. Bernard GR, Vincent J-L, Laterre P-F, LaRosa SP, Dhainaut J-F, Lopez-Rodriguez A, et al. Efficacy and safety of recombinant human activated protein c for severe sepsis. New Engl J Med. 2001;344:699–709.
19. Dhainaut JF, Yan SB, Joyce DE, Pettila V, Basson B, Brandt JT, et al. Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation. J Thromb Haemost. 2004;2:1924–33.
20. Arishima T, Ito T, Yasuda T, Yashima N, Furubeppu H, Kamikokuryo C, et al. Circulating activated protein C levels are not increased in septic patients treated with recombinant human soluble thrombomodulin. Thromb J. 2018;16:24.
21. Macias WL, Dhainaut JF, Yan SC, Helterbrand JD, Seger M, Johnson G 3rd, et al. Pharmacokinetic-pharmacodynamic analysis of drotrecogin alfa (activated) in patients with severe sepsis. Clin Pharmacol Ther. 2002;72:391–402.
22. Dhainaut JF, Yan SB, Margolis BD, Lorente JA, Russell JA, Freebairn RC, et al. Drotrecogin alfa (activated) (recombinant human activated protein C) reduces host coagulopathy response in patients with severe sepsis. Thromb Haemost. 2003;90:642–53.
23. Umemura Y, Yamakawa K, Ogura H, Yuhara H, Fujimi S. Efficacy and safety of anticoagulant therapy in three specific populations with sepsis: a meta-analysis of randomized controlled trials. J Thromb Haemost. 2016;14:518–30.
24. Okabayashi K, Wada H, Ohta S, Shiku H, Nobori T, Maruyama K. Hemostatic markers and the sepsis-related organ failure assessment

score in patients with disseminated intravascular coagulation in an intensive care unit. Am J Hematol. 2004;76:225–9.
25. Fourrier F, Chopin C, Goudemand J, Hendrycx S, Caron C, Rime A, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest. 1992;101:816–23.
26. Kinasewitz GT, Yan SB, Basson B, Comp P, Russell JA, Cariou A, et al. Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism. Crit Care. 2004;8:R82–90.
27. Esmon CT, Xu J, Lupu F. Innate immunity and coagulation. J Thromb Haemost. 2011;9(Suppl 1):182–8.
28. Ito T. PAMPs and DAMPs as triggers for DIC. J Intensive Care. 2014;2:67.
29. Conway EM, Van de Wouwer M, Pollefeyt S, Jurk K, Van Aken H, De Vriese A, et al. The lectin-like domain of thrombomodulin confers protection from neutrophil-mediated tissue damage by suppressing adhesion molecule expression via nuclear factor kappaB and mitogen-activated protein kinase pathways. J Exp Med. 2002;196:565–77.
30. Abeyama K, Stern DM, Ito Y, Kawahara K, Yoshimoto Y, Tanaka M, et al. The N-terminal domain of thrombomodulin sequesters high-mobility group-B1 protein, a novel antiinflammatory mechanism. J Clin Invest. 2005;115:1267–74.
31. Ito T, Kawahara K, Okamoto K, Yamada S, Yasuda M, Imaizumi H, et al. Proteolytic cleavage of high mobility group box 1 protein by thrombin-thrombomodulin complexes. Arterioscler Thromb Vasc Biol. 2008;28:1825–30.
32. Shi CS, Shi GY, Hsiao HM, Kao YC, Kuo KL, Ma CY, et al. Lectin-like domain of thrombomodulin binds to its specific ligand Lewis Y antigen and neutralizes lipopolysaccharide-induced inflammatory response. Blood. 2008;112:3661–70.
33. Ito T, Kakihana Y, Maruyama I. Thrombomodulin as an intravascular safeguard against inflammatory and thrombotic diseases. Expert Opin Ther Targets. 2016;20:151–8.
34. Hoffman M, Monroe DM 3rd. A cell-based model of hemostasis. Thromb Haemost. 2001;85:958–65.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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