Circulating Activated TAFI is a Prognostic Indicator in Septic DIC

Takaaki Totoki (tttkak@gmail.com)
Department of Anesthesiology, Fukuoka University School of Medicine

Takashi Ito
Kagoshima University Graduate School of Medical and Dental Sciences

Midori Kakuuchi
Kagoshima University Graduate School of Medical and Dental Sciences

Nozomi Yashima
Kagoshima University Graduate School of Medical Dental Sciences

Ikuro Maruyama
Kagoshima University Graduate School of Medical and Dental Sciences

Yasuyuki Kakihana
Kagoshima University Graduate School of Medical and Dental Sciences

Research

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Abstract

Background: Administration of recombinant human soluble thrombomodulin (rTM) is often used in Japan to treat septic disseminated intravascular coagulation (DIC). Thrombin-activatable fibrinolysis inhibitor (TAFI) is a fibrinolysis inhibitor activated by the thrombin-thrombomodulin complex, however, it is unknown whether circulating activated TAFI is increased after rTM administration in patients with DIC. Furthermore, the relationship between TAFI activation and the prognosis of septic DIC is not defined yet.

Objective: We sought to investigate the effect of rTM on TAFI activation and the association of plasma activated TAFI (TAFIa/ai) levels with the prognosis of septic DIC.

Methods: Using plasma samples from clinical studies conducted from May 2016–March 2017 on eight patients with septic DIC at Kagoshima University Hospital, we measured plasma levels of total TAFI, TAFIa/ai, thrombin-antithrombin complex (TAT), prothrombin fragment 1 + 2 (F1+2), soluble fibrin (SF), antithrombin (AT), protein C (PC), protein S (PS), and plasminogen activator inhibitor-1 (PAI-1) before and after intravenous rTM administration. Then, we evaluated the relationship of these marker levels to prognosis.

Results: The thrombin-rTM complex activated TAFI in vitro in plasma from a healthy volunteer. However, TAFIa/ai levels did not significantly increase over baseline in the septic DIC patients after intravenous rTM administration. Baseline TAFIa/ai levels in non-survivors were significantly higher than those in survivors.

Conclusions: Plasma TAFIa/ai did not increase with rTM administration. Elevated baseline TAFIa/ai concentration may be a negative prognostic indicator in septic DIC. Larger studies are needed to confirm the in vivo effect of rTM on TAFI activation.

Introduction

Thrombin-activatable fibrinolysis inhibitor (TAFI) is synthesized and secreted by the liver [1]. TAFI can be activated by thrombin, and this reaction is markedly promoted by thrombomodulin, an anticoagulant protein expressed on the surface of endothelial cells [2]. During fibrinolysis, plasmin partially hydrolyzes fibrin, and plasminogen, plasmin, and tissue-type plasminogen activator (tPA) bind to the C terminal lysine residue generated in partially-hydrolyzed fibrin. Activated TAFI inhibits the binding of plasminogen, plasmin, and tPA by selectively excising the lysine residue at the C-terminal of fibrin, thereby suppressing the fibrinolytic reaction and controlling the rate of fibrinolysis [3-5].

In sepsis, the expression of endothelial anticoagulant proteins, including thrombomodulin, is suppressed by endothelial damage. The decrease in thrombomodulin expression in the endothelium results in a hypercoagulable state and sepsis-associated disseminated intravascular coagulation (DIC) [6]. In Japan, recombinant human soluble thrombomodulin (rTM) is commonly used in expectation of activated protein C (APC)-dependent anticoagulant effects to counteract the hypercoagulable state in septic DIC [7].
Theoretically, administration of rTM may not only promote thrombin-mediated protein C activation but also promote thrombin-mediated TAFI activation, however, activated TAFI levels after rTM administration is unknown. Furthermore, the relationship between TAFI activation and the prognosis of septic DIC is not defined yet. In this study, we analyzed activated TAFI levels after rTM administration in patients with septic DIC and compared these levels in survivors and non-survivors.

Patients And Methods

Patients

The study was conducted using plasma samples from eight patients with septic DIC in our prior research on activated protein C conducted May 2016–March 2017 in Kagoshima University Hospital [8].

This prospective observational study conformed to the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of Kagoshima University Hospital. Between May 2016–March 2017, written informed consent was obtained from eight patients with sepsis-associated DIC prior to participation.

The diagnosis of sepsis and DIC was made according to the Third International Consensus Definition for Sepsis (Sepsis-3) [9] and the diagnostic criteria established by the Japanese Association for Acute Medicine (JAAM DIC criteria) [10], respectively.

Sample preparation for TAFI assays

Plasma from a healthy volunteer was incubated for 10 minutes at 37°C with varying concentrations of human thrombin (Sigma-Aldrich, St. Louis, MO, USA) and rTM (Asahi Kasei Pharma, Tokyo, Japan). The reaction was terminated by addition of a protease inhibitor cocktail containing hirudin (Sekisui Medical, Tokyo, Japan). The samples were centrifuged at 4°C and the supernatants were stored at −80°C until analysis of TAFI concentrations.

Blood samples collected from the eight patients with sepsis-associated DIC before and after administration of rTM (Asahi Kasei Pharma, Tokyo, Japan), (130 or 380 U/kg, depending on renal function) via intravenous drip infusion on day 1 and day 2 were immediately anticoagulated with a one-tenth volume of sodium citrate and kept at 4°C. The samples were then centrifuged at 2000 × g for 10 min at 4°C and plasma samples were stored at −80°C until analysis of TAFI concentrations.

Measurement of plasma levels of total TAFI, activated TAFI, TAT, F1+2, SF, AT, PC, PS, sTM, and PAI-1

Plasma total TAFI levels were analyzed by the Imuclone Total TAFI ELISA (Sekisui Diagnostics, Stamford CT, USA). Plasma activated TAFI levels (TAFIa/ai) were analyzed by the Asserachrom TAFIa/TAFIai (Diagnostica Stago, Seine, France). Plasma thrombin-antithrombin complex (TAT) levels were analyzed using Stacia chemiluminescence enzyme immunoassay (CLEIA) TAT (LSI Medience, Tokyo, Japan). Plasma prothrombin fragment 1+2 (F1+2) levels were analyzed using Enzygnost F1 + 2 (Siemens
Healthcare Diagnostics, Tokyo, Japan). Plasma soluble fibrin (SF) levels were analyzed using Iatron SF II (LSI Medience). Antithrombin (AT) was analyzed by a synthetic chromogenic substrate method using HemosIL ATLQ (Instrumentation Laboratory, Bedford MA, USA). Plasma protein C (PC) levels were measured by a synthetic chromogenic substrate method using HemosIL Protein C (Instrumentation Laboratory). Plasma protein S (PS) levels were measured by a synthetic chromogenic substrate method using HemosIL PS clot (Instrumentation Laboratory). Plasminogen activator inhibitor-1 (PAI-1) was analyzed using an LPIA • tPAI test (LSI Medience). All of the preceding assays were performed according to the manufacturers’ instructions.

Statistical analysis

For assessment of clinical samples, paired $t$-tests were used for comparisons before and after rTM treatment. Student $t$-tests were used for comparisons of the poor-survival group and the survival group. Data are presented as mean ± SD. A $p < 0.05$ was considered significant.

Results

As shown in Fig.1, activated TAFI levels were not increased when normal plasma samples were treated with 1 μg/mL of rTM in the absence of thrombin. In contrast, activated TAFI levels were increased when normal plasma samples were treated with the mixture of 0.1-0.5 U/mL of human thrombin and 1 μg/mL of rTM. Next, we examined the plasma levels of total TAFI, TAFIa/ai, F1+2, SF, AT, PS, and PAI-1 in eight patients with sepsis-associated DIC before and after administration of rTM over 2 days (Fig. 2).

The background characteristics of the eight patients are shown in Supplementary Table 1. Plasma soluble TM levels rapidly increased during a 30–60-min period of rTM administration (380 U/kg except for cases #3 and #5) to reach around 1 μg/mL [8]. In these conditions, plasma TAFIa/ai levels were not increased after rTM treatment except for the cases #3 and #8.

Then, we divided the eight consented patients into a poor-survival group and a survival group, and compared the measurements (Fig. 3). Plasma TAFIa/ai levels were significantly higher in the non-survival group compared with the survival group ($P < 0.05$). The other measurements were not significantly different between the poor-survival and survival groups.

Discussion

In this study, we found that thrombin-rTM promoted TAFI activation in vitro, but did not increase plasma TAFIa/ai levels in most patients with sepsis-associated DIC. Three (#3, #6, and #8) out of eight patients showed high total TAFI levels before rTM administration, and two (#3 and #8) out of the three patients showed high thrombin generation as evidenced by high F1+2 values. TAFIa/ai levels were increased in these two patients (#3 and #8) after rTM administration, suggesting that TAFIa/ai levels could be increased after rTM administration in patients with high total TAFI levels and high thrombin generation.
However, this hypothesis is based on the data of eight patients, and thus larger scale analysis is necessary to confirm it.

Extensive activation of TAFI has been reported as an independent predictor of mortality in sepsis [14]. Our findings support this hypothesis in patients with sepsis-associated DIC. Increased TAFIa/ai may cause ischemic organ failure by inhibiting intravascular fibrinolysis. Increased TAFIa/ai may be the result of increased thrombin generation, which is also thought to be associated with ischemic organ failure and poor outcome. So, extensive activation of TAFI may be the cause or the result of critically ill conditions. In the latter case, rTM administration can improve outcome by suppressing thrombin generation. In the former case, rTM administration may not worsen outcome because rTM administration did not increase activated TAFI levels in most cases. However, it should be noted that activated TAFI levels can be increased after administration of rTM if baseline levels of total TAFI and thrombin generation are simultaneously elevated. Previous studies showed that inhibition or knockout of TAFI alleviated sepsis-induced organ injury in mice [15-16], indicating that increased TAFIa/ai may account at least in part for sepsis-induced organ failure.

Conclusions

Plasma TAFIa/ai did not increase with rTM administration. Elevated baseline TAFIa/ai concentration may be a negative prognostic indicator in septic DIC. Larger studies are needed to confirm the in vivo effect of rTM on TAFI activation.

Abbreviations

AT: antithrombin; DIC: Disseminated intravascular coagulation; ELISA: Enzyme-linked immunosorbent assay; FDP: Fibrin/fibrinogen degradation products; F1 + 2: Prothrombin fragment 1 + 2; PAI-1: plasminogen activator inhibitor-1; rTM: Recombinant human soluble thrombomodulin; SF: soluble fibrin; sTM: soluble thrombomodulin; TAFI: thrombin-activatable fibrinolysis inhibitor; TAFIa: Activated thrombin activatable fibrinolysis Inhibitor; TAT: Thrombin-antithrombin complex

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Kagoshima University Hospital. Written informed consent was obtained from all patients prior to participation in the study.

Consent for publication

Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

IM and TI have received research funding from Asahi Kasei Pharma. All other authors state that they have no conflict of interests.

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**Authors’ contributions**

TT contributed to data analysis. TI contributed to study design and manuscript preparation. MK and NY contributed to in vitro experiments. IM and YK contributed to data analysis and manuscript editing. All authors read and approved the final manuscript.

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Figures
Figure 1

rTM promotes thrombin-mediated TAFI activation in vitro in plasma from a healthy volunteer. Plasma from a normal volunteer was incubated without and with thrombin (0–0.5 U/mL) and rTM (0–1 μg/mL) at 37°C for 10 min. rTM promoted TAFI activation only in the presence of thrombin. The data represent mean ± SD (n = 3).
Figure 2

Changes in coagulation/fibrinolysis markers in patients with sepsis-associated DIC before and after rTM administration. Blood samples were collected from the eight patients with sepsis-associated DIC before and after administration of rTM on day 1 and day 2. (A) Prothrombin fragment 1 + 2 (F1+2), (B) soluble fibrin (SF), (C) antithrombin (AT), (D) protein S (PS), (E) plasminogen activator inhibitor-1 (PAI-1), (F) total thrombin-activatable fibrinolysis inhibitor (TAFI), and (F) activated TAFI (TAFIa/ai) levels were analyzed according to the manufacturers’ instructions. TAFIa/ai levels were not increased after rTM treatment except for the case #3 and #8. In the case of #8, TAFIa/ai levels at any points were higher than 200
ng/mL, the upper limit of this measurement, however, the absorbance values were increased after rTM treatment from 2.19 to 2.32 on day 1, and from 2.48 to 2.62 on day 2.
Figure 3

Comparison of baseline coagulation/fibrinolysis markers in survivors and non-survivors. (A) Thrombin-antithrombin complex (TAT), (B) prothrombin fragment 1 + 2 (F1+2), (C) soluble fibrin (SF), (D) antithrombin (AT), (E) protein C (PC), (F) protein S (PS), (G) soluble thrombomodulin (sTM), (H) plasminogen activator inhibitor-1 (PAI-1), (I) total thrombin-activatable fibrinolysis inhibitor (TAFI), and (J) activated TAFI (TAFIa/ai) levels before rTM administration on day 1 in non-survivors (n = 3) were compared with those in survivors (n = 5). TAFIa/ai levels were significantly higher in non-survivors, and did not increase further with rTM administration. t–test was used for comparison of the poor-survival group and the survival group. *p < 0.05
Supplementary Files

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- SupplementaryTable1.docx
- TAFIcoverletter.TJ2020.docx