Antithrombin (AT) and Recombinant Human Thrombomodulin (rhTM) Combination as New Therapeutic Breakthrough in Managing Sepsis-Induced Coagulopathy (SIC)

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ABSTRACT Sepsis-induced coagulopathy (SIC) is a unique and urgent condition characterized by coagulation activation, overexpression of fibrinolysis, and high risk of organ dysfunction following sepsis. SIC is one of the complications of sepsis that is leading to a high mortality rate. Because there are no exact effective therapeutic agents specifically designed for SIC, new modalities are needed regarding the potential effect to suppress the mortality rate. Antithrombin (AT) and recombinant thrombomodulin (rhTM) are drugs which can promote anticoagulant activity. These natural anticoagulants work complementary to each other so that may suppress damage-associated molecular patterns (DAMPs) which is a process that may trigger endogenous molecule worsening the SIC. AT and rhTM have similar clinical effect on the other hand this AT-rhTM combination works on different sites and targets in the process. AT-rhTM combination may also suppress leukocyte adhesion and the elevation of circulating levels of the nucleosome, which leads to cell death event. Unfortunately, AT-rhTM combination costs extremely expensive. This article is done by searching journals with keywords "antithrombin", "thrombomodulin", and "sepsis-induced coagulopathy" on the search engines. From 55 journals that were reviewed, 46 were found suitable as a reference for this paper. AT-rhTM agent on SIC may reduce the mortality rate following some mechanisms. Further researches are required to identify the efficacy and effectivity of the AT-rhTM combination in treating patients with SIC.

KEYWORDS AT-rhTM combination, mortality, SIC

Introduction

Sepsis is an extreme and emergency condition of the human body characterized by the presence of simultaneous activation of inflammation and coagulation in response to microbial infection.[1] Sepsis explained as a systemic inflammatory response which often fatal and presenting a major public health problem.[2] It spent more than 5.2% of total United States hospital costs in 2011, which is more than USD 20 billion.[4] It is estimated that sepsis predisposes around 1.5 million people in the United States each year, affecting the rate of mortality to 250,000 individuals and is proven to be the major causes for 1 out of every three hospital deaths.[3]

The evolution related to its definition and management is correlated with many guidelines that have been published.[1,2] In 1980, description of sepsis was a systemic inflammatory host response to a microbial pathogen. In 2016, the third sepsis consensus conference defined sepsis as a 'dysregulated host-response' to infection leading to 'life-threatening organ dysfunction'.[5]

The mortality rate of sepsis is high because the complications

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that it creates such as shock, acute respiratory distress syndrome (ARDS), developed organ failure complications, and disseminated intravascular coagulation (DIC). Around 35% of sepsis cases are complicated by DIC or usually called Sepsis-induced coagulopathy (SIC).[1,6]

SIC is a condition when there are impairments of fibrinolysis roles, the activation of coagulation, and the downregulation of anticoagulant pathways.[7] Because DIC may worsen the condition that may lead to organ dysfunction, urgent treatment, and diagnostic tools are needed.[8] Pharmacological treatment of DIC is not recommended in 2012 guidelines of the Surviving Sepsis Campaign.[6] The recommendations for thrombosis in 2012 guideline of the Surviving Sepsis Campaign were to use daily pharmacological prophylaxis against venous thromboembolism, then patients with sepsis need to be treated with recombinant of pharmacological therapy and intermittent pneumatic compression devices. Lastly, it suggested using a mechanical prophylactic treatment such as graduated compression stockings or intermittent compression devices.[9]

There are no exact diagnostic tools or criteria have been designed for SIC; the majority of the health provider will give antithrombin (AT) as the main baseline therapy for patients with SIC.[10] However, Japan is encouraging treatment for SIC; Japan is one of the countries that use a combination of AT and recombinant human thrombomodulin (rhTM) to treat SIC, which showed a positive result in the process.[11]

In this paper, the author will discuss the therapeutic and potential combination of AT and rhTM in treating patients with SIC.

Review Method

The writing methodology used was a literature review. The source of literature included of relevant journals from the search engines www.pubmed.com, nature.com and scholar.google.com. Writers searched with keywords "antithrombin", "thrombomodulin", and "sepsis-induced coagulopathy" on the search engines. The inclusion criteria was that of all AT-rhTM combination therapy for SIC, and the preferred materials should not exceed the last ten years unless no newer study argues against the content. From 55 journals that were reviewed, 46 were found suitable as a reference for this paper. The collected information noted and analyzed for validity and reliability, interpreted and compiled into one scientific literature review.

Result and Discussion

The Pathogenesis of Sepsis

Sepsis is a complex clinical syndrome that occurs as a response to infection.[1] The contribution of apoptosis or programmed cell death is a pathophysiological process that has an important role in forming organ dysfunction following the imbalance in proinflammatory and anti-inflammatory cytokines. Until now, many types of research have provided information at a molecular, cellular, and organ levels.[12]

Innate immunity is the first step that embarks the host response to fight the pathogen, including the role of macrophages, monocytes, neutrophils, and natural killer cells.[13] This process happens through via the binding of pathogen-associated molecular patterns (PAMPs) including a component of bacterial, fungal, and viral pathogens.[14] Damage-associated molecular patterns (DAMPs) is another source of interaction that may release endogenous molecules from the damaged host cell, such as ATP, mitochondrial DNA.[14,15]

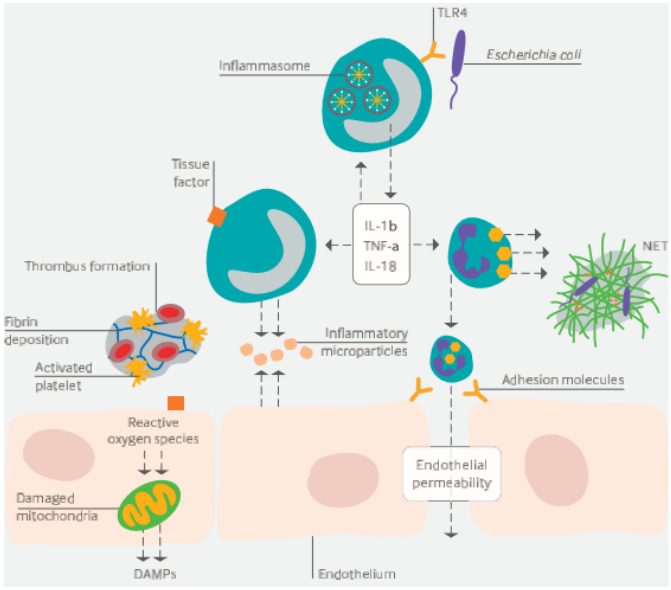

Figure 1: Overview of the pathogenesis of sepsis (1).[14] (1) Pathophysiologic processes in sepsis, including endothelial injury, endothelial barrier breakdown, immunothrombosis, and disseminated intravascular coagulation. DAMPs= damage associated molecular patterns; IL= interleukin; TLR4= Toll-like receptor 4; TNF-α=tumor necrosis factor α.

Such kind of mechanism may transduce and initiate transcription by binding to specific receptors.[14] Toll-like receptors (TLRs) is an example because it may recognize the membrane of bacterial that are expressed on the cell surface.[14-16] The other receptors like C-type leptin receptors, NOD-like receptors (nucleotide-binding oligomerization domain) and RIG-1 like receptors are also may induce the production of proinflammatory cytokines such as interleukin-1α (IL-1α), IL-1β, IL-6, and tumour necrosis factor-α (TNF-α).[13-17] These proinflammatory cytokines may initiate the activation of the innate or the adaptive immune response, which further will be shown in the increasing level of immunoregulatory or effector cytokine.[18]

The coagulation cascade is also affected for the reason of depression in anticoagulant effects of protein C and AT.[13] Thrombomodulin which itself is activated by thrombin converts protein C to its active form (activated protein C/APC) which may utilize anticoagulant effect and also be potent anti-inflammatory effects via the inhibition of TNFα, IL-1β, and IL-6 and limiting of neutrophil and monocyte adhesion to endothelium.[13-19] In patients with sepsis, there is the downregulation of thrombomodulin at the endothelial surface and impairment in synthesizing protein C because of proinflammatory cytokines.[20] Inflammatory cytokines decrease the issuance of the fibrinolytic pathway, which is shown by the increasing activity of plasminogen activator inhibitor 1 (PAI-1) and decreasing activity of plasmin. This activity leads to microvesicles that may result in exaggeration of inflammation and thrombosis.[21]

The Pathogenesis of Sepsis-induced Coagulopathy

DIC is a coagulation system disorder which happened because of the imbalance between clotting and fibrinolytic mechanisms. In some clinical condition, DIC developed by simultaneously

activated coagulation cascade mechanism to prevent excessive blood loss, followed by fibrinolytic pathway, and the overconsumption of coagulopathy finally may result in clotting which causes organ damage and failure.[22] DIC develops secondary to a clinical disorder, which makes a key for the proper examination and management.

One of the clinical spectrums includes sepsis.[23] During sepsis, either the cell membrane components of the microorganism or bacterial exotoxin produce proinflammatory cytokines, and the coagulation system will be diffusely activated.[6,22] The production of monocyte chemoattractant protein 1 and IL-6 in monocytes, fibroblasts, and mesothelial cells, are elicited by thrombin. Not only does thrombin affect those cytokines, but also the production of IL-6 and IL-8 in vascular endothelial cells by interacting with protease-activated receptors (PARs) 1, 3, and 4. factor Xa and the tissue factor-VIIa complex upregulate IL-6 and IL-8 in vascular endothelial cells through PAR2.[6,23] Furthermore, the causative agent and inflammatory response drive a simultaneously acting mechanism which is up-regulation of procoagulant pathways, down-regulation of physiological anticoagulants and suppression of fibrinolysis, leads to fibrin formation.[6,24-26]

In general, fibrin formation is followed by activation of fibrinolysis, which depends on PAI-1, thrombin-activatable fibrinolysis inhibitor (TAFI), including many such kinds of factors related to the underlying disease and the capability of the regulatory mechanism. However, in shortage of fibrinolysis leads to obstruction of the microvasculature.[26,27] Therefore, sepsis can embark the imbalance level of homeostasis, which may lead to the development of SIC.[27]

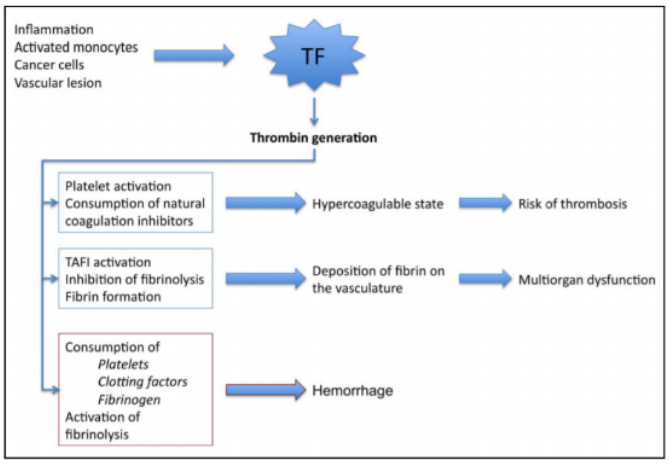

Figure 2: PPathways and clinical manifestations in DIC (2).[27] (2) Coagulation activity is controlled by TF overexpression, leading to consumption of natural coagulation inhibitors (mainly AT and PC) and in a hypercoagulable state. TAFI inhibits fibrinolysis increases fibrin formation and deposition in the microvasculature. AT= antithrombin; DIC= disseminated intravascular coagulation; PC= protein C; TF= tissue factor.

Overview of Antithrombin (AT)

AT is a heparin cofactor which composed of 432 amino acids and a member of the serine protease inhibitor family or also called as serpin. AT is classified in a range from I to IV, but these various AT have the same function as antithrombin III, which now called as AT.[28,29] AT is an anticoagulant and anti-inflammatory properties that restore homeostasis in the human body. [29]

AT works mostly by binding to activated factor II (thrombin) and factor Xa. Other than that, AT also neutralizes factors IXa, XIa and XIIa. Thrombin will cleave the reactive centre of antithrombin, that will trap the thrombin molecule and make an enzyme-inhibitor complex that excreted out of the circulation. [28,29]

AT as an anti-inflammatory property is mediated by its anticoagulation action. By blocking thrombin that activated platelets to secrete cytokines and stimulate leukocyte activity, AT contributes to decreasing inflammatory process in circulation. It inhibits the interaction between P-selectin, neutrophils and upregulates TF expression that may increase thrombin generation. However, AT also inhibits factor Xa that induced IL-6, IL-8, E-selectin that involved in pro-inflammatory factors. [29]

The use of antithrombin in treatment of SIC is still controversial. The only country that used AT as medicine for sepsis patients in Japan, where another country still use conservative treatment. Through trials such as KyberSept and PROWESS stated that use of AT or any anticoagulant agent in sepsis patients shown no significant effect and increase bleeding risks.[10] Japan adopted data from the trials that patients with SIC risks of mortality were decreased. While Kybersept trials used high-dosed of AT around 30000 IU for four days, Japan only uses 1500 U/day to 3000 IU/day for three days.[10,30] AT is used to treat patients with DIC whose antithrombin is less than 70%. Patients with DIC also need an evaluation of AT levels before and after administration of the AT.[30] Other than that, high dosed AT in KyberSept trials increase bleeding risk up to 20%, while medication for SIC in Japan claimed the risk of bleeding in SIC is only 5%.[10]

Overview of Recombinant Thrombomodulin (TM)

Thrombomodulin (TM) is a cell surface-expressed transmembrane glycoprotein localized primarily to the vascular endothelium, that integrates crucial biological processes and biochemical pathway.[31,32] Composition of the active extracellular domain of thrombomodulin is called rhTM.[33] Not only does thrombomodulin inhibits thrombin but also accelerating APC generation.[33] Thrombomodulin inhibits coagulation and inflammation, including the function of thrombin, such as fibrinogen clotting, platelet and EC activation, and Factor V activation.[33,34]

Not only does thrombomodulin have anticoagulant properties, but also anti-inflammatory properties. Thrombomodulin may exert its anti-inflammatory effect through APC-dependent and APC-independent mechanisms. APC acts on PAR-1 by cleaving the extracellular portion of PAR-1 to trigger the intracellular signalling pathways through endothelial cells and leukocytes.[35] It induces anti-inflammatory and cytoprotective effects.[31] Extracellular histones which play a role as mediators of endothelial dysfunction, organ failure and death in septic condition can be degraded by APC.[36]

APC-independent actions contribute directly in anti-inflammatory effect correlates with three mechanisms, PAMP, DAMP, and high mobility group Box 1 protein (HMGB1).[35] Modulating complements is one of the abilities that thrombomodulin has, thrombomodulin could inhibit the activation of the complement from classical and lectin pathways.[41] These functions result in the suppression of C3a and C5a complements that induce excessive inflammatory responses.[35,37] Inhibition of leukocyte necrosis was shown by the administration of rhTM

followed by omitting the circulating DAMPs, suppressing the coagulation responses and showing capability in the organ protection.[38] Also, the suppressed endotoxin-induced inflammatory responses by thrombomodulin can be triggered in several ways. For example, the lectin-like domain of thrombomodulin binding to endotoxin and the competency in neutralizing endotoxin-induced inflammatory responses.[35]

Reconstruction and administration of antithrombin (AT)-recombinant human thrombomodulin (rhTM) combination

Combination of Antithrombin (AT) and recombinant human Thrombomodulin (rhTM)

There are many natural anticoagulants known to diminish the death associated with sepsis, which the most popular and novel one is the combination of AT and rhTM.[39] In order to get the soluble form of thrombomodulin, it is isolated so that extracellular domains is the only compartment left. rhTM then can be administered intravenously given the dose of 0.06 mg kg-1 for 30 min once a day.[40] While antithrombin will be given in the dose of 1500 U/day to 3000 IU/day for three days.[10,30] There were many studies that failed, revealing the clinical effect of this combination.[39] Both of AT and rhTM plays an important role in the coagulation and inflammatory system. AT itself has the most important inhibition role in the coagulation system, it may inhibit the thrombin activation up to 80% and many other coagulation factors such as IIa, IXa, Xa, and XIIa.[39,41] rhTM also works by complementing the inhibition effect against the coagulation process. rhTM may activate protein C to alter the thrombogenicity of thrombin, which inhibits factors Va and VIIa.[39,40] The coagulation factors target and sites of action are different results in independent inhibitory action at different sites. AT binds to receptors on the endothelial cells while rhTM works are inhibiting coagulation in the circulation.[39,42]

Necrotic leukocytes inhibition reduce the circulating DAMPs, thereby easing the coagulation process and improving organ protection.[39] AT-rhTM combination may also suppress leukocyte adhesion and the elevation of circulating levels of nucleosome which reflecting the cell death event. Not only does the combination can suppress leukocyte adhesion but also the leukocyte-endothelial interaction, thereby lowering the HMGB1 level.[39] HMGB1 suppression indicates the lesser inflammation process through shrinkage of immune responses.[43]

The ex-vivo experiment also confirmed the supporting theory about this therapeutic combination. By using fluorescent staining, necrosis significantly suppressed, which means the combination may successfully decrease the circulating DAMPs such as histone H3 and cf-DNA.[39]

Beneficial Analysis of AT-rhTM combination for sepsis-induced coagulopathy

Significantly improvement was found that AT-rhTM combination therapy improved platelet counts and D-dimer levels compared with AT monotherapy in patients with severe sepsis and DIC.[44] Coadministration of AT and rhTM are improving the several markers as stated before after the injection of Lipopolysaccharide (LPS) in endotoxic rats.[39,45] Moreover, I also found that a combination of AT-rhTM therapy has improvements in coagulation parameters, organ failure rates, and survival rates, compared with AT monotherapy.[46]

Limitations of AT-rhTM Combination for Sepsis-Induced Coagulopathy (SIC)

There are some limitations despite the benefits of AT-rhTM combination. One of the shortages is the lack of a valid diagnostic tool for SIC. Coagulation activation with over suppression of fibrinolysis and a high incidence of organ dysfunction is a typical characteristic of SIC. One of the most challenging things about the procedure is the cost, antithrombin therapy for three days will cost approximately USD 2000 and six days of treatment with rhTM will cost almost USD 4000.[10] More studies related to bleeding complications should also be performed to know whether modality affects the risk of bleeding.[10,44]

Conclusions

The current studies about the combination of AT-rhTM showing great potential therapeutic strategy in treating SIC. The combination itself offers two helpful mechanisms in terms of the coagulation factor target and different sites of action, which makes the independent inhibitory action and also suppress the inflammation that are induced by sepsis. Some studies show this therapeutic agent has a significant beneficial effect in reducing the mortality risks. On the other hand, some still shows no improvement findings related to the many factors that can affect the outcome, such as age and severity of sepsis. Since then, the effectiveness of this new agent should be repeatedly evaluated.

Further studies of clinical trials are required to clarify the exact clinical effects since the benefits of this combination therapy remain unclear. More studies are also necessary to acquaint the mechanism of this modality.

Competing Interests

The authors declared that this review was done independently without any conflict of interest of any organizations that would lead this review to bias.

References

1. Polat G, Ugan R, Cadirci E, Halici Z. Sepsis and Septic Shock: Current Treatment Strategies and New Approaches. The Eurasian Journal of Medicine. 2017;49(1):53-58.

2. Keeley A, Hine P, Nsutebu E. The recognition and management of sepsis and septic shock: a guide for non-intensivists. Postgraduate Medical Journal. 2017;93(1104):626-634.

3. Hajj J, Blaine N, Salavaci J, Jacoby D. The "Centrality of Sepsis": A Review on Incidence, Mortality, and Cost of Care. Healthcare. 2018;6(3):90.

4. Singer M, Deutschman C, Seymour C, Shankar-Hari M, Annane D, Bauer M et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801.

5. Mira J, Gentile L, Mathias B, Efron P, Brakenridge S, Mohr A et al. Sepsis Pathophysiology, Chronic Critical Illness, and Persistent Inflammation-Immunosuppression and Catabolism Syndrome. Critical Care Medicine. 2017;45(2):253-261

6. Okamoto K, Tamura T, Sawatsubashi Y. Sepsis and disseminated intravascular coagulation. Journal of Intensive Care. 2016;4(1).

7. Tsao C, Ho S, Wu C. Coagulation abnormalities in sepsis. Acta Anaesthesiologica Taiwanica. 2014;53:16-22.

8. Iba T, Nisio M, Levy J, Kitamura N, Thachil J. New criteria for sepsis-induced coagulopathy (SIC) following the revised sepsis definition: a retrospective analysis of a nationwide survey. BMJ Open. 2017;7(9):e017046.

9. Dellinger R, Levy M, Rhodes A, Annane D, Gerlach H, Opal S et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. Intensive Care Medicine. 2013;39(2):165-228.

10. Thachil J, Iba T. The application of anticoagulant therapy to sepsis. Journal of Intensive Care. 2017;5(1).

11. Hayakawa M. Management of disseminated intravascular coagulation: current insights on antithrombin and thrombomodulin treatments. 2018;10:25-29.

12. Nagar H, Piao S, Kim C. Role of Mitochondrial Oxidative Stress in Sepsis. Acute and Critical Care. 2018;33(2):65-72.

13. Chang J. Sepsis and septic shock: endothelial molecular pathogenesis associated with vascular microthrombotic disease. Thrombosis Journal. 2019;17(1).

14. Gotts J, Matthay M. Sepsis: pathophysiology and clinical management. BMJ. 2016;:i1585.

15. Gyawali B, Ramakrishna K, Dhamoon A. Sepsis: The evolution in definition, pathophysiology, and management. SAGE Open Medicine. 2019;7:205031211983504.

16. Deng M, Scott M, Loughran P, Gibson G, Sodhi C, Watkins S et al. Lipopolysaccharide Clearance, Bacterial Clearance, and Systemic Inflammatory Responses Are Regulated by Cell Type-Specific Functions of TLR4 during Sepsis. The Journal of Immunology. 2013;190(10):5152-5160.

17. Chaudhry H, Zhou J, Zhong Y, et al. Role of cytokines as a double-edged sword in sepsis. In Vivo. 2013;27(6):669–684.

18. Schulte W, Bernhagen J, Bucala R. Cytokines in Sepsis: Potent Immunoregulators and Potential Therapeutic Targets—An Updated View. Mediators of Inflammation. 2013;2013:1-16.

19. Yamakawa K, Murao S, Aihara M. Recombinant Human Soluble Thrombomodulin in Sepsis-Induced Coagulopathy: An Updated Systematic Review and Meta-Analysis. Thrombosis and Haemostasis. 2018;119(01):056-065.

20. King E, Bauzá G, Mella J, Remick D. Pathophysiologic mechanisms in septic shock. Laboratory Investigation. 2013;94(1):4-12.

21. Hotchkiss R, Moldawer L, Opal S, Reinhart K, Turnbull I, Vincent J. Sepsis and septic shock. Nature Reviews Disease Primers. 2016;2(1).

22. Venugopal A. Disseminated intravascular coagulation. Indian Journal of Anaesthesia. 2014;58(5):603.

23. Strande J, Phillips S. Thrombin increases inflammatory cytokine and angiogenic growth factor secretion in human adipose cells in vitro. Journal of Inflammation. 2009;6(1):4.

24. Levi M, Schultz M, van der Poll T. Disseminated Intravascular Coagulation in Infectious Disease. Seminars in Thrombosis and Hemostasis. 2010;36(04):367-377.

25. Levi M. The Coagulant Response in Sepsis. Clinics in Chest Medicine. 2008;29(4):627-642.

26. Semeraro N, Ammollo C, PAPLI F, Colucci M. SEPSIS-ASSOCIATED DISSEMINATED INTRAVASCULAR COAGULATION AND THROMBOEMBOLIC DISEASE. Mediterranean Journal of Hematology and Infectious Diseases. 2010;2(3):e2010024.

27. 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. Clinical and Applied Thrombosis/Hemostasis. 2018;24(9_suppl):8S-28S.

28. Hepner M, Karlaftis V. Antithrombin. Haemostasis. 2013;992:355-364.

29. Sniecinski R, Welsby I, Levi M, Levy J. Antithrombin: anti-inflammatory properties and clinical applications. Thrombosis and Haemostasis. 2016;115(04):712-728.

30. Tagami T. Antithrombin concentrate use in sepsis-associated disseminated intravascular coagulation: re-evaluation of a 'pendulum effect' drug using a nationwide database. Journal of Thrombosis and Haemostasis. 2018;16(3):458-461.

31. Loghmani H, Conway E. Exploring traditional and nontraditional roles for thrombomodulin. Blood. 2018;132(2):148-158.

32. Li Y, Kuo C, Shi G, Wu H. The role of thrombomodulin lectin-like domain in inflammation. Journal of Biomedical Science. 2012;19(1):34.

33. Tagami T, Matsui H, Fushimi K, Yasunaga H. Use of Recombinant Human Soluble Thrombomodulin in Patients with Sepsis-Induced Disseminated Intravascular Coagulation after Intestinal Perforation. Frontiers in Medicine. 2015;2.

34. Bongoni A, Klymiuk N, Wolf E, Ayares D, Rieben R, Cowan P. Transgenic Expression of Human Thrombomodulin Inhibits HMGB1-Induced Porcine Aortic Endothelial Cell Activation. Transplantation. 2016;100(9):1871-1879.

35. Ito T, Kakihana Y, Maruyama I. Thrombomodulin as an intravascular safeguard against inflammatory and thrombotic diseases. Expert Opinion on Therapeutic Targets. 2015;20(2):151-158.

36. Silk E, Zhao H, Weng H, Ma D. The role of extracellular histone in organ injury. Cell Death & Disease. 2017;8(5):e2812-e2812.

37. Tateishi K, Imaoka M, Matsushita M. Dual modulating functions of thrombomodulin in the alternative complement pathway. BioScience Trends. 2016;10(3):231-234.

38. Iba T, Miki T, Hashiguchi N, Tabe Y, Nagaoka I. Combination of antithrombin and recombinant thrombomodulin modulates neutrophil cell-death and decreases circulating DAMPs levels in endotoxemic rats. Thrombosis Research. 2014;134(1):169-173.

39. Iba T, Miki T, Hashiguchi N, Yamada A, Nagaoka I. Combination of antithrombin and recombinant thrombomodulin attenuates leukocyte–endothelial interaction and suppresses the increase of intrinsic damage–associated molecular patterns in endotoxemic rats. Journal of Surgical Research. 2019;187;581-586.

40. Ikezoe T. Thrombomodulin/activated protein C system in septic disseminated intravascular coagulation. Journal of Intensive Care. 2015;3(1).

41. Iba T, Thachil J. Present and future of anticoagulant therapy using antithrombin and thrombomodulin for sepsis-associated disseminated intravascular coagulation: a perspective from Japan. International Journal of Hematology. 2015;103(3):253-261.

42. Chappell D, Jacob M, Hofmann-Kiefer K, Rehm M, Welsch U, Conzen P et al. Antithrombin reduces shedding of the endothelial glycocalyx following ischaemia/reperfusion. Cardiovascular Research. 2009;83(2):388-396.

43. Wang H, Ward M, Sama A. Targeting HMGB1 in the treatment of sepsis. Expert Opinion on Therapeutic Targets. 2014;18(3):257-268.

44. Yasuda N, Goto K, Ohchi Y, Abe T, Koga H, Kitano T. The efficacy and safety of antithrombin and recombinant human thrombomodulin combination therapy in patients with severe sepsis and disseminated intravascular coagulation. Journal of Critical Care. 2016;36:29-34.

45. Iba T, Nakarai E, Takayama T, Nakajima K, Sasaoka T, Ohno Y. Combination effect of antithrombin and recombinant thrombomodulin in a lipopolysaccharide induced rat sepsis model. Crit Care 2009;13, R203. [12]

46. Sawano H, Shigemitsu K, Yoshinaga Y, Tsuruoka A, Natsukawa T, Hayashi Y, et al. Combination therapy with antithrombin and recombinant human soluble thrombomodulin in patients with severe sepsis and disseminated intravascular coagulation. J Jpn Assoc Acute Med 2013;24:119–31.