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Review Article

The roles of activated protein C in experimental trauma models

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ABSTRACT

Trauma-induced coagulopathy is classified into primary and secondary coagulopathy, with the former elicited by trauma and traumatic shock itself and the latter being acquired coagulopathy induced by anemia, hypothermia, acidosis, and dilution. Primary coagulopathy consists of disseminated intravascular coagulation and acute coagulopathy of trauma shock (ACOTS). The pathophysiology of ACOTS is the suppression of thrombin generation and neutralization of plasminogen activator inhibitor-1 mediated by activated protein C that leads to hypocoagulation and hyperfibrinolysis in the circulation. This review tried to clarify the validity of activated protein C hypothesis that constitutes the main pathophysiology of the ACOTS in experimental trauma models.

Introduction

Trauma-induced coagulopathy has been classified into primary and secondary coagulopathy, with the former is elicited by trauma and traumatic shock itself and the latter being acquired coagulopathy induced by anemia, hypothermia, acidosis, and dilution (Table 1).[1] For more than half a century, primary coagulopathy has been considered to be disseminated intravascular coagulation (DIC)[2]; however, in 2007, Brohi et al.[3,4] created a new disease entity called acute coagulopathy of trauma-shock (ACOTS) and claimed that there has been nothing to suggest the existence of DIC.[5] Interestingly, they recently changed their claims, resulting in ACOTS being regarded as having almost the same pathophysiology as DIC.[6,7] In the present review, we tried to clarify the validity of the activated protein C (APC) hypothesis that constitutes the main pathophysiology of ACOTS in experimental trauma models.

Although other terms such as “acute coagulopathy of trauma” or “acute traumatic coagulopathy” etc. are now used to describe ACOTS, the original term created by the advocates, ACOTS, is mostly used in this review.

Pathophysiology of DIC

The main characteristics of DIC are systemic thrombin generation due to the activation of the tissue factor-induced coagulation pathway and insufficient anti-coagulation mechanisms, such as tissue factor pathway inhibitor (TFPI), antithrombin, protein C and endothelial thrombomodulin due to endothelial injury, and suppression of fibrinolysis by plasminogen activator inhibitor-1 (PAI-1).[1] DIC is usually considered to be the thrombotic disease according to such changes as the activation of coagulation, insufficient anti-coagulation and suppression of fibrinolysis, however, when DIC and such conditions as shock-induced hypoperfusion or systemic ischemia and hypoxia, which enhance systemic fibrin(ogen)olysis, coexist with DIC, then DIC with the fibrinolytic phenotype ensues due to tissue-type plasminogen activator (t-PA) release from Weibel-Palde bodies in the endothelium.[1,2]

APC hypothesis

APC plays central roles in the ACOTS, which occurs only in patients with traumatic shock with severe metabolic acidosis. Shock-induced hypoperfusion slows the clearance of thrombin from the circulation. Both newly expressed endothelial thrombomodulin and soluble thrombomodulin generated in the circulation with full domains and activity complex with the thrombin. Thrombin and thrombomodulin complexes then form systemic APC converted

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Table 1
The classification of trauma-induced coagulopathy.

Trauma-induced coagulopathy
1. Physiological changes
• Hemostasis and wound healing
2. Pathological changes
• Endogenously induced primary pathologies
- Disseminated Intravascular Coagulation (DIC)
Activation of coagulation
Insufficient anticoagulant mechanisms
Increased fibrin(ogen)olysis (early phase)
Suppression of fibrinolysis (late phase)
- Acute coagulopathy trauma-shock (ACOTS)*
APC-mediated suppression of coagulation
APC-mediated increased fibrinolysis
• Exogenously induced secondary pathologies that modify DIC and ACOTS
- Anemia-induced coagulopathy
- Hypothermia-induced coagulopathy
- Acidosis-induced coagulopathy
- Dilutional coagulopathy
- Others

* ACOTS is referred to by various names including (but not limited to) acute traumatic coagulopathy and acute coagulopathy of trauma, etc. Some researchers refer to ACOTS as trauma-induced coagulopathy. APC: activated protein C.

from protein C.[3–5] APC inactivates activated Factors Va and FVIIIa (FVa and FVIIIa), thereby leading to the systemic suppression of thrombin generation. Furthermore, APC neutralize PAI-1, which induces an increase in the "production" of t-PA.

The differences in pathophysiology of DIC and APC hypothesis in ACOTS are shown in Fig. 1.[2] Points meriting discussion are systemic thrombin generation, anticoagulant mechanisms, endothelial injury, and fibrinolytic systems under an ACOTS condition.

We searched the PubMed database for *in vivo* experimental studies in the English language, published from January 2007 to May 2017 using key words "protein C", "thrombin", "procoagulant", "coagulopathy", "plasminogen activator inhibitor-1", "fibrinolysis", "disseminated intravascular coagulation" "trauma", "injuries". Eight of 2600 studies met the inclusion criteria and were narratively reviewed.

APC and thrombin generation

A mouse model of trauma and hemorrhagic shock confirmed the activation of the tissue factor-dependent coagulation pathway, leading to increases in the levels of both APC and thrombin and antithrombin complex (TAT), a marker of thrombin generation.[8] Parallel increases in the APC and TAT were noted.

Traumatic brain injury and hemorrhagic shock in a porcine model brought about immediate increases in the levels of prothrombin fragment 1 + 2 (PF1+2), a marker of thrombin generation, with no simultaneous changes in the APC levels. This increase persisted throughout the study period, with increases in the levels of APC observed at post 2 h from the induction of hemorrhagic shock.[9] Another study confirmed significant increases in the levels of soluble fibrin, a marker of thrombin generation, and its direct action to fibrinogen, without any changes in the levels of APC immediately after Noble-Collip drum shock in rats.[10] Furthermore, this group demonstrated spontaneous thrombin burst in the thrombin generation assay, which clearly indicated the presence of systemic circulating procoagulants. The results coincide with the findings of two human studies, in which the authors confirmed the same phenomenon; trauma patients showed excessive non-wound-related thrombin generation and tissue factor-like activity in the systemic circulation.[11,12]

In a rat polytrauma model, the measured APC levels were at the lower detection limit of the assay.[13] In contrast, the thrombin levels gradually increased with parallel decreases in the levels of prothrombin, suggesting that thrombin was being produced, which leads to the consumption of prothrombin.[13] In addition, an *in vitro* study confirmed that elevated APC levels failed to reach a concentration that was high enough to suppress thrombin generation via the inactivation of platelets and plasma FVa.[14] Another *in vitro* study confirmed that 300–2000 ng/mL of APC was needed to suppress the activities of FV and FVIII, and to prolong both the prothrombin time (PT) and activated partial prothrombin time (APTT).[15] These levels were extremely high compared with those observed in clinical studies (5–65 ng/mL), showing that the APC-mediated suppression of thrombin generation in ACOTS is unlikely.[16–18]

APC and anticoagulant mechanisms and endothelial injury

Increases in the levels of APC at 2 h after traumatic brain injury and hemorrhagic shock were associated with endothelial activation, confirmed by increases in the levels of syndecan-1, von Willebrand factor and soluble vascular cell adhesion molecue-1.[9] An ovine model

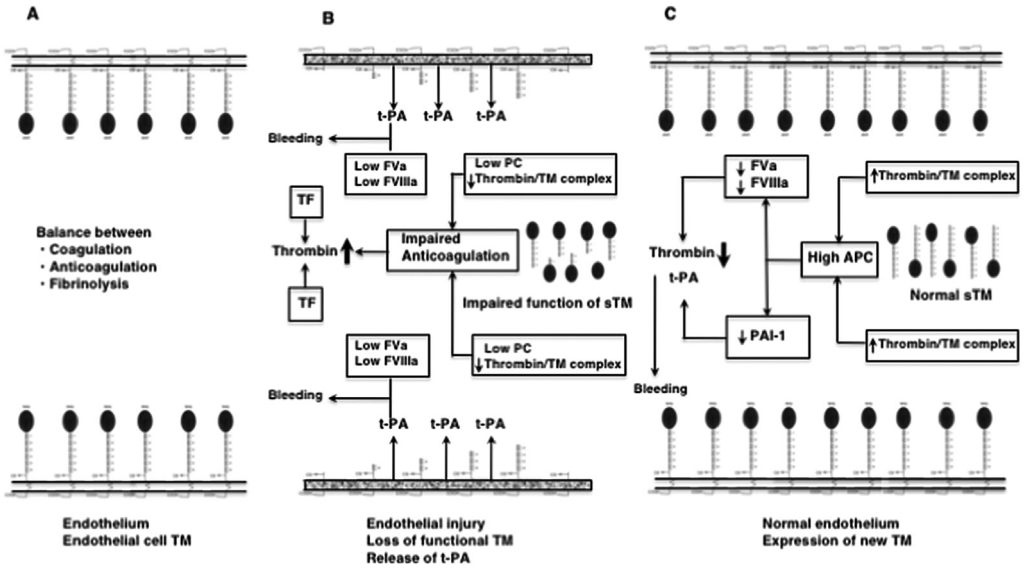

Fig. 1. The Pathophysiology of DIC with the fibrinolytic phenotype and APC hypothesis in ACOTS. A: normal coagulation and fibrinolysis; B: DIC with the fibrinolytic phenotype; C: ACOTS. TM: thrombomodulin; sTM: soluble TM; TF: tissue factor; PC: protein C: APC: activated protein C; t-PA: tissue-type plasminogen activator.

Table 2
Summary of *in vivo* experimental studies.

Study (year)	Animal	Experiment	Sampling time *(n)*	APC	Thrombin (surrogate)	PAI-1	Main results
Chesebro et al[24] (2009)	Mouse	Control Trauma (laparotomy), T Hemorrhagic shock (MAP, 65 mmHg, 60min), H Trauma/hemorrhagic shock, TH	After 60 min (1)	Yes	No	No	TH mice had an elevated APTT and increased APC levels. The selective inhibition of the anticoagulant property of APC by monoclonal antibodies prevented the prolongation of APTT in response to TH. The blockade of both the anticoagulant and cytoprotective function of APC caused 100% mortality with histopathological findings of pulmonary thrombosis and perivascular and alveolar hemorrhage.
Hayakawa et al[20] (2013)	Rat	Control Tissue factor 4 U/kg infusion, low dose Tissue factor 16 U/kg infusion, high dose	Immediately 2 h 4 h (3)	No	No	No	High-dose tissue factor caused increases in PAP, D-dimer, FDP and FgDP levels, which were associated with decreased α2-plasmin inhibitor and fibrinogen levels. These changes were accompanied by lower platelet counts, prolonged PT, and decreased antithrombin levels.
Sillesen et al[9] (2014)	Swine	Control Traumatic brain injury and hemorrhagic shock (MAP, 30 −35 mmHg, 120min), TBI/H	Baseline 3 min post injury 15 min post injury 2 h after shock (4)	Yes	No	Yes	The TBI/H group showed immediate increases in PF1+2 and a marker of endothelial activation (syndecan-1), which continued 2 h post-shock. However, increases in APC levels were observed at 2 h after hemorrhagic shock; this was not associated with significant changes in the D-dimer and PAI-1 levels, but was associated with significant increases in the PF1+2 levels.
Hayakawa et al[10] (2015)	Rat	Control Noble-Collip drum trauma, NCD Trauma0, blood sample drawn immediately after NCD Trauma30, blood sample drawn 30 min after NCD	Immediately after NCD 30 min after NCD (2)	Yes	Yes	No	NCD caused no changes in the APC levels. Trauma 0 and 30 were both associated with increases in soluble fibrin, sTM, active t-PA, D-dimer, FDP, FDP/D-dimer ratio and FgDP. These changes were accompanied by decreases in platelet counts, fibrinogen, antithrombin, Factors II, V, and VIII activities and the prolongation of PT. Spontaneous thrombin bursts were observed in Trauma0 in a non-stimulated thrombinogram. The peak height/FII and endogenous thrombin potential/FII ratios were negatively correlated with the antithrombin levels
Howard et al[8] (2015)	Mouse	Sham Trauma (laparotomy) + hemorrhagic shock (MAP, 35 ± 5 mmHg, 60min), TH TH + IgG TH + anti-tissue factor antibody TH + saline TH + hirudin	After 60 min (1)	Yes	Yes	No	TH caused parallel increases in both TAT and APC. Anti-tissue factor antibodies and hirudin blocked these increases.
Wu et al[13] (2016)	Rat	Poly trauma and hemorrhage	Before trauma 30, 60, 120, and 240 min after trauma (5)	Yes	Yes	Yes	The increases in APC was not significant and the measured levels were at the lower limits of the assay. Thrombin activity was preserved. Antithrombin and α2-macroglobulin fell within 2 h and the sTM was elevated for over 4 h. The plasmin activity was elevated for the entire 4 h, however, the t-PA level was elevated at 30 min, then decreased, while the D-dimer levels increased at 4 h. The PAI-1 levels increased at 2−4 h. The APC did not inhibit the increase in PAI-1.
van Zyl et al[19] (2016)	Ovine	Control Moderate trauma with 20% volume hemorrhage, M Severe trauma with 30% volume hemorrhage, S	Baseline 30min, 1,3,5 h after injury (5)	Yes	Yes	No	Protein C decreased with elevated levels of both APC and sTM from 3 h. Factors V and VIII decreased from 1 h to 3 h, respectively. PAI-1 was reduced from 30 min after injury, but no changes in the D-dimer levels were observed throughout the experiment. These results were obtained only from severe trauma.
Davenport et al[7] (2017)	Mouse	Tauma/hemorrhagic shock (MAP, 25−30 mmHg, 60min), TH Wild type, WT TM knockin, TMKI Homozygos FV Leiden	Baseline After 60 min experimental period (2)	Yes	No	No	TH increased APC in WT mice but this increase was attenuated in TMKI mice. The increases in the D-dimer levels in WT were reduced in TMKI mice. The study showed no results in relation to the APC-mediated suppression of thrombin generation and degradation of PAI-1.

APC: activated protein C; APTT: activated partial thromboplastin time; FDP: fibrin/fibrinogen degradation products; FgDP: fibrinogen degradation products; MAP: mean arterial pressure; PAI-1: plasminogen activator inhibitor-1; PAP: plasmin and α2 plasmin inhibitor complex; PF1+2: prothrombin fragment 1 + 2; PT: prothrombin time; sTM: soluble thrombomodulin; TAT, thrombin antithrombin complex; t-PA, tissue-type plasminogen activator.
Note: Yes means that the parameter is measured in the studies and No means that the parameter is not measured in the studies.

of trauma and hemorrhage also showed that increases in the levels of APC were associated with increased levels of syndecan-1, hyaluranon, and soluble thrombomodulin, suggesting glycocalyx degradation, endothelial activation and injury.[19] Furthermore, two rat trauma models demonstrated significant and immediate decreases in antithrombin activity and increases in levels of soluble thrombomodulin, a direct marker of endothelial injury.[10,13] One study further showed decreases in the levels of α2-macroglobulin.[13] A significant decrease in antithrombin and α2 macroglobulin, two major anticoagulant factors, suggested their binding to thrombin and the neutralization of thrombin action. However, glycocalyx degradation and endothelial injury suppressed the anticoagulant actions of these factors, leading to systemic thrombin generation, irrespective of APC dynamics.[10,13]

Hayakawa et al.[10] showed significant negative correlations between the antithrombin activity and endogenous thrombin potential/FII and peak height/FII ratios using a thrombin generation assay. Their findings clearly indicated the insufficient control of thrombin generation by antithrombin, leading to systemic thrombin generation measured by soluble fibrin.[10] Dunbar et al.[11] also demonstrated significant negative correlations between antithrombin activity and the tissue factor-stimulated termination time ratio using the same method, suggesting that reduced antithrombin levels allow for systemic thrombin generation. They further confirmed the systemic thrombin generation in patients with acute coagulopathy of trauma.[11]

These results indicate that impaired anticoagulant mechanisms and endothelial injury can induce systemic thrombin generation in a pathological state that overwhelms the APC-mediated inhibition of thrombin generation observed in physiological hemostasis at the injured site.

APC and fibrinolysis

The Noble-Collip drum shock trauma model in rat demonstrated immediate and significant increases in active t-PA that were unable to be inactivated by PAI-1 in association with systemic fibrin(ogen) olysis as confirmed by increases in the levels of fibrinogen/fibrin degradation products (FDP), D-dimer, and FDP/D-dimer ratios.[10] A Western blot analysis in this experiment showed a clear increase in fibrinogen degradation products (FgDP) immediately to 30 min after trauma. Of note, these changes were observed without any changes in the levels of APC. The same group further demonstrated that massive amounts of tissue factor induce fibrinolysis and fibrinogenolysis in parallel with increases in the levels of plasmin and α2-plasmin inhibitor complex (PAP), a marker of plasmin generation, without tissue hypoperfusion.[20] These changes were associated with consumption coagulopathy, namely decreases in the platelet counts, fibrinogen, antithrombin, and α2-plasmin inhibitor, and prolonged PT.

Using a rat polytrauma model, Wu et al.[13] showed that an early increase in active t-PA drives the elevation of plasmin activity with no changes in the APC levels. A late increase in active PAI-1 with low t-PA levels suggested the induction and expression of PAI-1 mRNA because this phenomenon usually takes several hours.[21,22] The time courses of t-PA and PAI-1 in this experimental model coincided with a change in DIC with the fibrinolytic to thrombotic phenotype; in the former type, an extreme imbalance between high t-PA and low PAI-1 plays an important role in critical bleeding at the early phase and persistently high PAI-1 levels in the latter type also play a role in thrombosis at the late phase of trauma.[1,22]

Significant elevation in the levels of APC at 3–5 h after trauma in an ovine model of trauma and hemorrhage failed to prove a PAI-1-mediated increase in fibrinolysis.[19] In that experiment, no marked changes in the D-dimer levels were noted, despite a decrease in the PAI-1 levels. The authors stated that this phenomenon is consistent with previously published results, in which APC cannot inhibit PAI-1 to the levels required to induce clinically relevant fibrinolysis.[23] This conflicts with the ACOTS theory.

Thrombomodulin-knock-in mice with a reduced capacity to APC that were subjected to traumatic hemorrhage showed significant attenuation of both increases in the D-dimer levels and decreases in the fibrinogen levels.[7] This transgenic mouse model also showed an improved median survival time in compared with wild-type mice after traumatic hemorrhage. These results may suggest that APC plays some roles in increased fibrinolysis after trauma; however, its relationship to PAI-1 and t-PA remains unclear at present.

APC and cytoprotection

Mice with trauma and hemorrhagic shock showed a significant increase in APC and a greater prolongation of APTT than controls and simple laparotomy mice.[24] Monoclonal antibody 1591 selectively inhibits the anticoagulant function of APC, which improves the prolongation of APTT; however, no effect on the APC level was noted in this experiment. Pretreatment with monoclonal antibody 1591 before trauma and hemorrhagic shock did not induce any pulmonary pathology; however, pretreatment with monoclonal antibody 1609, which inhibits both the anticoagulant and cytoprotective functions of APC, induces pulmonary artery thrombosis, and perivascular and alveolar hemorrhage. The results suggest that the cytoprotective function of APC may be necessary to inactivate coagulation systems.

A summary of the *in vivo* experimental studies cited in this review is shown in Table 2.

The major limitations of the present review are that it was a narrative review and that the results obtained were entirely limited to data from very small experimental studies. In parallel with this review, we conducted systematic review on the same subject using clinical studies.[25] The conclusion of the systematic review of clinical studies was that, “APC plays no major roles in the inhibition of coagulation or increased fibrinolysis in ACOTS”. This was similar to the present review of experimental studies. A systematic review to investigate the roles of APC in trauma in both experimental and clinical studies is warranted.

Conclusion

Experimental trauma models described in this review failed to show direct evidence of APC-operated suppression of thrombin generation and enhancement of t-PA increase due to PAI-1 neutralization. This indicates that the APC hypothesis in ACOTS is unlikely in experimental models. Other mechanisms underlying increase in fibrinolysis due to APC remains to be elucidated.

Ethical approval and consent for publication

Not applicable for this study.

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Author's contributions

All the three authors conducted to conception and design of this review. Gando S wrote this review and Mayumi T and Ukai T supervised all processes of this review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cjtee.2018.07.005.

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