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 (Table 1). For more than half a century, primary coagulopathy has been considered to be disseminated intravascular coagulation (DIC); 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. Interestingly, they recently changed their claims, resulting in ACOTS being regarded as having almost the same pathophysiology as DIC[^5]. In the present review, we tried to clarify the validity of activated protein C hypothesis that constitutes the main pathophysiology of the ACOTS in experimental trauma models.

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**Introduction**

Trauma-induced coagulopathy has been classified into primary and secondary coagulopathy, with the former being 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-Palade bodies in the endothelium.[^4,^5]

**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...
from protein C. 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 leads to the consumption of prothrombin. 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. 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). 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.

**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 molecule-1. An ovine model

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**Table 1**

The classification of trauma-induced coagulopathy.

| Type of Coagulopathy | Conditions | Other Pathologies |
|----------------------|------------|------------------|
| **Acute Coagulopathy** | Hemostasis and wound healing | Endogenously induced primary pathologies |
| **Disseminated Intravascular Coagulation (DIC)** | Activation of coagulation | Insufficient anticoagulant mechanisms |
| | Insufficient fibrin(ogen)olyis | Increased fibrin(ogen)olysis (early phase) |
| | Insufficient plasminogen activator (PAI-1) | Suppression of fibrinolysis (late phase) |
| | Insufficient endogenous anticoagulant mechanisms | APC-mediated suppression of coagulation |
| | Insufficient endogenous fibrinolytic mechanisms | APC-mediated increased fibrinolysis |

* 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.

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**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 (2009) | Mouse | Control Trauma (laparotomy), T Hemorrhagic shock (MAP, 65 mmHg, 60 min), 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 (2013) | Rat | Control | Tissue factor 4 U/kg infusion, low dose | Immediately 2 h | No | No | No | High-dose tissue factor caused increases in PAP, D-dimer, FDP and FgDP levels, which were associated with decreased s2-plasmin inhibitor and fibrinogen levels. These changes were accompanied by lower platelet counts, prolonged PT, and decreased antithrombin levels. |
| Sillesen et al (2014) | Swine | Control | Traumatic brain injury and hemorrhagic shock (MAP, 30–35 mmHg, 120 min), TBI/H | Baseline | 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. |
| Howard et al (2015) | Mouse | Sham | Trauma (laparotomy) + hemorrhagic shock (MAP, 35 ± 5 mmHg, 60 min), TH | 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/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. |
| Wu et al (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 s2-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 (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 (2017) | Mouse | Trauma/hemorrhagic shock (MAP, 25–30 mmHg, 60 min), TH Wild type, WT TM knockin, TMKI Homozygous FV Leiden | Baseline | 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 s2 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, hyalur- 
anon, and soluble thrombomodulin, suggesting glycolalyx degra-
dation, endothelial activation and injury.19 Furthermore, two rat 
trauma models demonstrated significant and immediate decreases 
in antithrombin activity and increases in levels of soluble thrombo-
modulin, a direct marker of endothelial injury.10,13 One study further 
showed decreases in the levels of α2-macroglobulin.11 A significant 
decrease in antithrombin and α2 macroglobulin, two major antico-
gulant factors, suggested their binding to thrombin and the 
neutralization of thrombin action. However, glycoalyx degradation 
and endothelial injury suppressed the anticoagulant actions of these 
factors, leading to systemic thrombin generation, irrespective of APC 
dynamics.20,21

Hayakawa et al.13 showed significant negative correlations be-
tween the antithrombin activity and endogenous thrombin poten-
tial/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 genera-
tion measured by soluble fibrin.12 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.13

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 fibrinogen 
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.13 These changes were 
associated with consumption coagulopathy, namely decreases in 
the platelet counts, fibrinogen, antithrombin, and α2-plasmin in-
hibitor, and prolonged PT.

Using a rat polytrauma model, Wu et al.13 showed that an early 
increase in active t-PA that 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.12,22

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 fibrino-
lysis.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 
atteuation 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 selec-
tively 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 cyto-
protective 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.

Fund

No funding or financial support was used for this study.

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 su-
pervised 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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