Antithrombin-III suppresses collagen-induced human platelets activation

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Introduction

Sepsis and Septic DIC

- Disseminated intravascular coagulation (DIC) is most common symptom observed in severe sepsis and septic shock.
- Sepsis-induced DIC is characterized by microvasucular thrombosis, reduction of platelets and coagulation inhibitors[1].

Platelet activation

- Collagen is one of most important stimuli for platelet activation.
- Activated platelets secrete platelet-derived growth factor (PDGF)-AB and release soluble CD40 ligands (sCD40L). PDGF-AB is a mitogenic mediator and plays a key role in the development of arteriosclerosis[2]. sCD40 ligand is one of the inflammatory mediators in atherosclerosis process[3].

Antithrombin

- Antithrombin (AT) is known as an important physiological anti-coagulant.
- AT inactivates thrombin and multiple other coagulation factors, thereby AT is used for anticoagulant therapy in sepsis-associated DIC treatment[4].

In our previous studies, we found that pretreatment of AT-III inhibited collagen-stimulated platelet aggregation, secretion of the PDGF-AB and release of sCD40L[5]. However, it is impossible to administrate AT-III before platelets activation as a treatment for septic coagulopathy.

Then, we investigated the effect of AT-III as a treatment for collagen-stimulated activated platelets.

Methods

Subject

- Twelve healthy volunteers, who have not been taking any antiplatelet medicines, were enrolled in this study.

Platelet preparation and measurement of platelet functions

- Blood was centrifuged to separate platelet rich plasma (PRP) and platelet poor plasma (PPP).
- PRP was stimulated by 0.5 µg/ml of collagen.
- After the PRP stimulation with collagen, AT-III was administrated at 15, 30, 45, 60, 90 or 120 sec later.
- Platelet aggregation was recorded by aggregometer.
- After recording platelet aggregation, PRP mixture was centrifuged.
- Then, the supernatant was subjected to ELISA for PDGF-AB and sCD40L.

Statistical analysis

- Data were analyzed by Student's t-test.

This study was approved by the ethics committee, and written informed consent was obtained.

Results

Representative data showing the effect of AT-III on the collagen-induced platelet aggregation

Black line indicates the percentage of transmittance of each sample (the isolated platelets were recorded as 0%, and PPP was recorded as 100%). Blue line indicates small aggregates (9-25 µm); green line, medium aggregates (25-50 µm); red line, large aggregates (50-70 µm).

Effect of AT-III on collagen-induced PDGF-AB secretion

AT-III administration within 45 sec after collagen stimulation significantly inhibited the secretion of PDGF-AB.

Effect of AT-III on collagen-induced sCD40L release.

AT-III administration within 60 sec after collagen stimulation significantly inhibited the release of sCD40L.

Discussion

AT-III administration after collagen stimulation significantly suppressed platelet aggregation, and also inhibited secretion of the PDGF-AB and release of sCD40L after platelet activation.

→ It is likely that the effect of AT-III administration suppressed platelets activation after 45-60 sec collagen stimulation.

AT is rapidly depleted in the early phases of sepsis[6]. AT-III treatment was reported to increase survival in sepsis, and AT might be efficacious in sepsis and septic shock[7-10].

→ Our results can provide a possible explanation of therapeutic effect of AT in sepsis.

We have previously reported suppressive effect of AT-III pretreatment on collagen-stimulated platelet activation [5]. In sepsis, all platelets will not be activated at once, but will be activated one after another. Therefore, taken together, AT-III therapy at early period from sepsis onset is considered to be more efficacious.

Conclusion

In septic coagulopathy, we suggest that administration of AT-III, just after platelet activation, may provide an anti-coagulant and anti-inflammatory effect. Starting of AT-III therapy as soon as possible after diagnosis of coagulopathy may be more effective.

References

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