



Title	Noble-Collip Drum Trauma Induces Disseminated Intravascular Coagulation But Not Acute Coagulopathy of Trauma-Shock
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**Title**

Noble–Collip drum trauma induces disseminated intravascular coagulation but not acute coagulopathy of trauma-shock

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**Short running head**

Blunt trauma induces DIC but not ACoTS

**Conflicts of Interest and Source of Funding**

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## **ABSTRACT**

**Background:** There are 2 opposing possibilities for the main pathogenesis of trauma-induced coagulopathy: an acute coagulopathy of trauma shock, and disseminated intravascular coagulation (DIC) with the fibrinolytic phenotype.

**Objective:** To clarify the main pathogenesis of trauma-induced coagulopathy using a rat model of Noble–Collip drum trauma.

**Methods:** Eighteen rats were divided into the Control, Trauma 0, and Trauma 30 groups. The Trauma 0 and 30 groups were exposed to Noble–Collip drum trauma. Blood samples were drawn without, immediately after, and 30 min after Noble–Collip drum trauma in the Control, Trauma 0, and Trauma 30 groups, respectively. Coagulation and fibrinolysis markers were measured. Thrombin generation was assessed according to a calibrated automated thrombogram.

**Results:** Spontaneous thrombin bursts resulting from circulating procoagulants were observed in the non-stimulated thrombin generation assay immediately after trauma. Soluble fibrin levels (a marker of thrombin generation in the systemic circulation) were 50 fold greater in the trauma groups than the Control group. The resultant coagulation activation consumed platelets, coagulation factors, and antithrombin. Endogenous thrombin potential and factor II ratio were significantly negatively correlated with antithrombin levels, suggesting insufficient control of thrombin generation by antithrombin. High levels of active tissue-type plasminogen activator induced hyper-fibrin(ogen)olysis. Soluble thrombomodulin increased significantly. However, activated protein C levels did not change.

**Conclusions:** The systemic thrombin generation accelerated by insufficient

antithrombin control leads to the consumption of platelets and coagulation factors associated with hyper-fibrin(ogen)olysis. These changes are collectively termed DIC with the fibrinolytic phenotype.

**Key words:** fibrinolysis, fibrinogenolysis, procoagulant, thrombin, fibrinolytic phenotype

**Abbreviations:**

ACoTS: acute coagulopathy of trauma-shock

DIC: disseminated intravascular coagulation

F: factor

FDP: fibrinogen/fibrin degradation products

FgDP: fibrinogen degradation products

ETP: endogenous thrombin potential

t-PA: tissue-type plasminogen activator

TIC: trauma-induced coagulopathy

## INTRODUCTION

Trauma-induced coagulopathy (TIC) is multifactorial, involving hemodilution, hypothermia, and traumatic coagulopathy caused by trauma and traumatic shock (1-3). A review published 5 years ago explains the main pathogenesis of TIC during the early phase of trauma as an acute coagulopathy of trauma-shock (ACoTS) induced by both tissue injury and traumatic shock (4-6); traumatic shock retards thrombin clearance, thus increasing its binding to endothelial thrombomodulin on the surface of endothelial cells and soluble thrombomodulin in the circulation (4-6). The formation of thrombin–thrombomodulin complexes consequently activates protein C, which inactivates factors (F)Va and FVIIIa (4-6). These processes shut down thrombin generation and induce bleeding tendency. Furthermore, these mechanisms are believed to be the pathogenesis of ACoTS (4-6).

In contrast, a Japanese research group claims disseminated intravascular coagulation (DIC) with the fibrinolytic phenotype and not ACoTS is the predominant mechanism underlying TIC (1-3). Tissue injuries caused by blunt trauma induce damage-associated molecular patterns in the systemic circulation and activate the tissue factor-dependent coagulation pathway followed by systemic thrombin generation (1-3). Systemic thrombin generation induces the consumption of coagulation factors as well as proteins that control coagulation, such as antithrombin and protein C (1-3). Low levels of antithrombin and protein C enhance uncontrolled coagulation activation in the systemic circulation, which is not restricted to the injured sites (1-3). These changes lead to the formation of fibrin thrombosis in the systemic circulation. Tissue

hypoperfusion resulting from both traumatic shock and microvascular fibrin thrombosis induces acute excessive release of tissue-type plasminogen activator (t-PA) from Weibel–Palade bodies in the endothelial cells (1-3,7); this induces fibrinogenolysis in addition to fibrinolysis, followed by the consumption of  $\alpha_2$ -plasmin inhibitor, which further enhances fibrin(ogen)olysis (1-3). All of these changes are believed to be involved in the pathogenesis of DIC with the fibrinolytic phenotype in the early phase of trauma (1-3).

Various animal models have been used to study the pathogenesis of TIC (8,9). However, previous experimental studies do not completely clarify the pathological mechanisms of the hemostatic changes during the early phase of trauma. The ideal model of TIC should incorporate significant tissue injuries and traumatic shock (8,9). Accordingly, the Noble–Collip drum-induced trauma and traumatic shock model was developed approximately 50 years ago (10); this severe blunt trauma model is used to mimic lethal traumatic injury by causing both massive tissue injuries and traumatic shock without gross hemorrhage. Thus, the present study aimed to clarify the main pathogenesis of TIC by using a rat model with Noble–Collip drum trauma.

## **MATERIALS AND METHODS**

### **Animals**

All animals were housed and treated in accordance with the Standards of Animal Experiments of Hokkaido University. All experiments were approved by the Institutional Ethical Review Board at Hokkaido University.

Male 9-week-old Wistar S/T rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). They were allowed to acclimate for a minimum of 1 day in our animal breeding quarters before being subjected to experimentation. The breeding quarters were maintained at 20°C, and the animals were fed a standard diet and given access to water ad libitum.

### **Experimental procedures**

Each rat was anaesthetized intraperitoneally with pentobarbital 30 mg/kg (Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan) and restrained in the supine position. The trachea and left carotid artery were exposed with a small incision. The rat was subsequently placed in a Noble–Collip drum (10), which is a plastic wheel 38 cm in diameter with internal shelves; it was rotated 500 times at 50 rpm with an anesthetized rat inside. During rotating of the wheel, the anesthetized rat was repeatedly struck down from the top of the inside of the wheel. The mortality rate of rats exposed to the Noble–Collip drum trauma model without treatment was 50% 2 h after trauma induction (unpublished results).

After the induction of Noble–Collip drum trauma, the left carotid artery was catheterized with a 24-gauge SURFLO (Terumo, Tokyo, Japan) catheter to enable mean arterial pressure monitoring and arterial blood sampling. Mean arterial pressure was monitored with a TruWave Disposable Pressure Transducer (Edwards Lifesciences, Irvine, CA, USA) and a Viridia component monitoring system (Hewlett–Packard Japan, Tokyo, Japan). To maintain arterial catheter patency, normal saline was constantly infused at 1 mL/h and tracheostomy was performed. During the experimental period, rectal



temperature was maintained at 37–39°C.

A total of 18 rats were randomly divided into 3 groups as follows: (a) the Control group was not exposed to Noble–Collip drum trauma, and arterial blood pressure was measured and arterial blood was drawn after anesthesia ( $n = 6$ ); (b) the Trauma 0 group was exposed to Noble–Collip drum trauma immediately followed by arterial blood pressure measurement and arterial blood sampling ( $n = 6$ ); and (c) the Trauma 30 group was exposed to Noble–Collip drum trauma followed by arterial blood pressure measurement at 0, 15, and 30 min and arterial blood sampling at 30 min ( $n = 6$ ). Blood (1 mL) was immediately placed into an exclusive vacuum blood collection tube for serum fibrin degradation products (FDP) (Venoject II, Terumo, Tokyo, Japan), which contained thrombin, aprotinin, and snake venom. The rest of the blood samples were immediately diluted with 4% sodium citrate (1:9 v/v). A portion of whole blood was used for arterial blood gas analysis and blood cell counts. The other samples were promptly separated by centrifugation for 5 min at 3000 rpm at 4°C, and serum and plasma were frozen at –80°C until analysis.

### **Measurements**

Arterial blood gas and lactate levels were analyzed by an ABL 700 (Radiometer, Copenhagen, Denmark). Plasma antithrombin and soluble fibrin levels as well as serum D-dimer and FDP levels were measured by an LPIA-NV7 (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Prothrombin time, coagulation factor activities, and fibrinogen levels were measured by an ACL Top coagulation analyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

FII, FV, FVII, and FVIII activities were measured by using human plasma with respective coagulation factor deficiency. The mean activity of each coagulation factor in the Control group was set at 100%. The antigen levels of soluble thrombomodulin and tissue factor were determined using the Rat Thrombomodulin (TM) ELISA Kit (CSB-E07939R, Cusabio Biotech Co., Ltd., Wuhan, China) and the Tissue Factor ELISA Kit, Rat (WLS-E90524RA, Usbn Life Science Inc., Wuhan, China). The antigen level of functionally active t-PA, which is not inactivated by plasminogen activator inhibitor, was determined using the Rat tPA Active ELISA Kit (IRTPAKT, Innovative Research, Inc., Novi, MI, USA). The antigen level of activated protein C was determined using the Activated Protein C ELISA kit, Rat (WLS-E90738Ra, Usbn Life Science Inc., Wuhan, China); the test principle employed by this kit is a sandwich enzyme immunoassay, which involves the use of an antibody specific to activated protein C.

### **Thrombin generation assay**

Thrombin generation was assessed using a calibrated automated thrombogram (Thermo Thrombinoscope, Finggal Link Co. Ltd., Tokyo, Japan). Stimulated thrombin generation was measured by the normal calibrated automated thrombogram method. Plasma samples (80  $\mu$ L) were supplemented with 20  $\mu$ L PPP-Reagent (Finggal Link), which contains a mixture of phospholipids and tissue factor. At the start of measurement, 20  $\mu$ L FluCa-Kit (Finggal Link), which contains HEPES (pH 7.35), calcium chloride, and fluorogenic substrate, was applied automatically to the plasma samples

supplemented with PPP-Reagent. Non-stimulated thrombin generation was measured using a modified calibrated automated thrombogram method: instead of PPP-Reagent, 80  $\mu$ L plasma was supplemented with 20  $\mu$ L Owren veronal buffer (OV-30, Sysmex Co., Kobe, Japan). At the start of measurement, 20  $\mu$ L FluCa-Kit was applied automatically to the plasma samples supplemented with Owren veronal buffer in the same manner as that for stimulated thrombin generation measurement. A FluoroScan Ascent fluorometer (Fingal Link) obtained measurements every 10 s, and the data were analyzed using Thrombinoscope software. To correct for inner-filter effects and substrate consumption, each measurement was corrected with respect to the fluorescence curve obtained from a mixture of the sample plasma with a fixed amount of thrombin- $\alpha_2$ -macroglobulin complex (Thrombin Calibrator, Fingal Link). The parameters calculated by the software included lag time, time to peak, peak height, and endogenous thrombin potential (ETP). All samples and calibrators were run at least in duplicate.

### **Measurement of fibrinogen degradation products (FgDP)**

FgDP were measured as described previously (11). Samples from the exclusive vacuum blood collection tubes for serum FDP were diluted with SDS sample buffer without a reducing agent. The diluted samples were boiled at 100°C for 5 min, loaded onto SDS-polyacrylamide gels (4–15% Mini-PROTEAN TGX gel, Bio-Rad Laboratories, Inc., Hercules, CA, USA), electrophoresed, and electrophoretically transferred to polyvinylidene-difluoride filter membranes. The membranes were incubated with the primary antibody (monoclonal rabbit

anti-fibrinopeptide A (EPR2919) antibody, Abcam, Tokyo, Japan) and subsequently incubated with the secondary antibody coupled to horseradish peroxidase. The membranes were visualized with an enhanced chemiluminescence detection kit (ECL Advance Western Blotting Detection Kit, GE Healthcare Japan, Tokyo, Japan) and a chemiluminescence imaging system (Light Capture II, ATTO, Tokyo, Japan). The captured images were analyzed by the Cool Saver analyzer (ATTO, Tokyo, Japan). The quantities of FgDP are expressed with respect to the density of the positive control, which was set at 100%.

### **Statistical analysis**

Unless otherwise indicated, all measurements are expressed as means  $\pm$  standard deviation (SD). Comparisons among the 3 groups were made using the Kruskal–Wallis test. The Mann–Whitney *U*-test with a Bonferroni correction was applied as a post hoc analysis (i.e., Control vs. Trauma 0 or Control vs. Trauma 30). Spearman rank correlation coefficients were calculated to determine correlations between antithrombin and the peak height/FII and ETP/FII ratios. SPSS 15.0J (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The level of statistical significance was set at  $P < 0.05$ .

## **RESULTS**

The general characteristics of each group are presented in Table 1. After the induction of Noble–Collip drum trauma, lactic acidosis and massive tissue

damage were observed, as indicated by elevated levels of creatine kinase and lactate dehydrogenase. Hypotension was observed just after the induction of blunt trauma (0 min), but blood pressure gradually returned to normal. The effect of hemodilution was excluded, because no fluid was administered.

Platelet counts, coagulation, and fibrinolytic variables are shown in Table 2. After the induction of blunt trauma, thrombin generation (observed as higher levels of soluble fibrin) and activity increased 50 fold compared to those in the Control group (Figure 1). Platelets, fibrinogen, various coagulation factors, and antithrombin were consequently consumed. Antigen levels of functional active t-PA, which cannot be inactivated by plasminogen activator inhibitor, increased slightly but significantly after trauma (Figure 2). Significant fibrin(ogen)olysis was confirmed by increases in D-dimer and FDP levels as well as the FDP/D-dimer ratio (Figure 2). Representative results of western blot analyses for FgDP are shown in Figure 3. Increased FgDP levels support the existence of fibrinogenolysis. Although soluble thrombomodulin levels increased significantly, there were no changes in activated protein C levels. Tissue factor was not detected in any samples.

The results of the thrombin generation assay are presented in Table 3. In the stimulated thrombin generation assay, lag time and time to peak were shorter in the Trauma 0 and 30 groups than the Control group. Furthermore, ETP decreased as a result of the consumption of coagulation factors. However, the peak height/FII and ETP/FII ratios, which are indexes of thrombin generation control, were significantly greater in the Trauma 0 and 30 groups than the Control group. Furthermore, antithrombin level was significantly negatively

correlated with peak height/FII ratio and ETP/FII ratio ( $\rho = -0.733$ ,  $P < 0.001$  and  $\rho = -0.839$ ,  $P < 0.001$ , respectively) (Figure 4). In the non-stimulated thrombin generation assay, spontaneous thrombin bursts were observed after blunt trauma induction. Representative thrombin generation curves in the Control and Trauma 0 groups are shown in Figure 5. The spontaneous thrombin burst observed in the Trauma 0 group did not occur in the Control group.

## DISCUSSION

The non-stimulated thrombin generation assay showed spontaneous thrombin bursts immediately after severe blunt trauma. This could be due to the activation of coagulation systems by circulating procoagulants released into the systemic circulation from massively injured tissues. Chandler et al. report the same phenomenon in patients with severe trauma (12,13); trauma patients exhibit unregulated coagulation activation characterized by excessive non-wound-related thrombin generation due to circulating procoagulants capable of activating systemic coagulation, which was also observed in the present study (12,13). Circulating procoagulants may include tissue factor, phospholipids, collagen, microparticles, and other substances released from injured tissues and activated cells including platelets, monocytes/macrophages, and endothelial cells (12-16). Tissue factor was not detected in any samples in the present study. However, previous studies indicate that the procoagulants might be circulating tissue factor (13,14). Thus, the present results might be attributable to the low sensitivity of the ELISA, which has a detection limit of 30

pg/mL. Furthermore, we were unable to specify the procoagulants involved. However, as they were detected in the systemic circulation within minutes after blunt trauma, they cannot be substances synthesized and released after injury.

Soluble fibrin is formed as a result of the direct action of thrombin on fibrinogen; it is used as a marker of thrombin generation and its activity (17). Soluble fibrin provides more direct evidence of thrombin activity than prothrombin fragment 1+2 or thrombin–antithrombin complex (17). Therefore, the extremely elevated soluble fibrin levels observed in the present study are direct evidence of thrombin generation in the systemic circulation. Several previous clinical investigations report coagulation activation immediately after trauma according to these markers (18-20). Elevated levels of these markers indicate systemic coagulation activation after trauma but do not directly indicate the presence of procoagulants in the systemic circulation. The results of the non-stimulated thrombin generation assay suggest the presence of circulating procoagulants and higher soluble fibrin levels, directly corroborating increased thrombin generation and its activity in the systemic circulation.

Platelet counts, fibrinogen levels, and the activities of coagulation factors decreased after Noble–Collip drum trauma. In addition, FII activity decreased remarkably within minutes after blunt trauma. FII, which is also called prothrombin, is a substrate of thrombin. Therefore, the decrease in FII levels may be due to the thrombin-induced consumption. Furthermore, marked decreases in FII, FV, and FVIII induced a decrease in ETP shown in the stimulated thrombin generation assay. The decreased platelet and fibrinogen levels can be explained through consumption but not depletion due to massive

bleeding and hemodilution, which are characteristics of the Noble–Collip drum trauma model. Thus, these results clearly support the pathophysiology of DIC, in which increased thrombin generation in the circulation and reduced hemostatic capacity caused by consumption coagulopathy outside of the vessels can be observed.

Antithrombin levels were markedly decreased immediately after blunt trauma the same as in previous clinical studies (12,13,18,21-23). The massive thrombin generation, which was observed as elevation of soluble fibrin, induces consumption of not only coagulation factor but also antithrombin. Furthermore, in the present study, augmentations in vascular permeability were observed as hemoconcentration (without significance, in Table 1). The augmentations in vascular permeability may induce antithrombin leakage, such as occurs in severe sepsis (24-25). The insufficient control of coagulation due to reduced antithrombin levels accelerates thrombin generation, which is not restricted to the injured sites. Although FII activity halved after trauma, the peak height/FII and ETP/FII ratios, which are indexes of thrombin generation control, increased significantly and were strongly negatively correlated with antithrombin levels. These results demonstrate thrombin generation in the circulation is accelerated by insufficient control of antithrombin despite decreased levels of FII, which is a substrate of thrombin. Dunbar et al. describe dysregulated thrombin generation induced by reduced antithrombin levels in trauma patients, which corroborates the present results (12).

Although the rat model described herein did not cause hemorrhagic shock with prolonged hypotension, severe tissue injuries and traumatic shock



induced marked elevation of lactate levels; this suggests extensive tissue hypoperfusion, which can cause the release t-PA from endothelial cells. Fibrin(ogen)olysis detected on the basis of elevated FDP and D-dimer levels and FDP/D-dimer ratio as well as direct evidence of fibrinogenolysis in western blot analysis clearly demonstrate the t-PA–induced activation of fibrinolytic systems. In addition, microvascular fibrin thrombosis-induced tissue hypoperfusion might contribute to t-PA release from endothelial cells.

In the main concept of ACoTS, thrombin–thrombomodulin complex activates protein C, which subsequently induces the shutdown of thrombin generation followed by bleeding tendency (4-6). In the present study, massive thrombin generation in the systemic circulation and extreme elevation of soluble thrombomodulin levels were confirmed after blunt trauma. Soluble thrombomodulin is a marker of endothelial injury, suggesting the impairment of endothelial thrombomodulin (26-28). Elevations of soluble thrombomodulin are observed in patients with not only trauma but also sepsis and out-of-hospital cardiac arrest (28-29). In addition, soluble thrombomodulin has only 20% of the activity of intact thrombomodulin because it is cleaved from intact thrombomodulin on endothelial cells (30). Various insults induce an endothelial injury and loss of thrombomodulin functions of endothelial cells (28-29). These changes resulted in the insufficient control of the inhibition of both tenase and prothrombinase complexes by activated protein C. Furthermore, no changes in activated protein C levels were detected after blunt trauma. These results strongly suggest that neither the concept nor pathological mechanisms of ACoTS are likely involved in the Noble–Collip drum trauma model.

There are 2 potential treatments for DIC with the fibrinolytic phenotype in the early phase of trauma. Plasma transfusion is one of the important treatments in the early phase of trauma (31). In this phase of trauma, consumption of both coagulation factor and anti-coagulation factors was observed. Administration of fresh frozen plasma, which contains both coagulation and anti-coagulation factors, can treat both the consumptive coagulopathy and dysregulation of thrombin generation. Another potential treatment for DIC with the fibrinolytic phenotype in the early phase of trauma is early administration of tranexamic acid. Tranexamic acid inhibits fibrin(ogen)olysis and improves bleeding tendency. In a large clinical trial, early administration of tranexamic acid improved outcomes in patients with severe bleeding (32). However, microvascular fibrin thrombosis may be increased by administration of tranexamic acid, because activation of thrombin generation in the systemic circulation is observed in the early phase of trauma,

In conclusion, rats subjected to blunt trauma and traumatic shock caused by the Noble–Collip drum exhibit a spontaneous thrombin burst due to the activation of the coagulation system by circulating procoagulants in the systemic circulation. In turn, systemic thrombin generation accelerates as a result of insufficient control of antithrombin. The activation of the coagulation system consumes platelets and coagulation factors, which results in reduced hemostatic potential outside the vascular system. Moreover, fibrin-induced secondary fibrinolysis and tissue hypoperfusion-induced t-PA release from the endothelial cells lead to hyper-fibrin(ogen)olysis. We term these changes “DIC with the fibrinolytic phenotype.” Meanwhile, there was no evidence of ACoTS,

particularly the shutdown of thrombin generation induced by the protein C–thrombomodulin pathway in the systemic circulation, although the present results were obtained in an animal study.

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## FIGURE LEGENDS

**FIGURE 1.** Changes in soluble fibrin levels after trauma. Soluble fibrin levels increased approximately 50 fold after blunt trauma in the Trauma 0 and 30 groups compared to the Control group ( $P = 0.02$ , Kruskal–Wallis test). \*  $P < 0.05$  vs. Control, Mann–Whitney  $U$ -test with Bonferroni correction.

**FIGURE 2.** Changes in the levels of functionally active t-PA, D-dimer and FDP, and FDP/D-dimer ratio after trauma. Functionally active t-PA levels increased significantly after trauma in the Trauma 0 and 30 groups ( $P = 0.0018$ , Kruskal–Wallis test). These changes suggest fibrinolysis and fibrinogenolysis along with the elevation of D-dimer and FDP levels, and FDP/D-dimer ratio ( $P < 0.001$ ,  $P < 0.001$ ,  $P = 0.028$ , respectively, Kruskal–Wallis test). \*  $P < 0.05$  vs. Control, Mann–Whitney  $U$ -test with Bonferroni correction.

**FIGURE 3.** FgDP. (A) Representative results of western blot analyses for FgDP in the 3 groups are shown. (B) FgDP levels increased significantly after trauma ( $P = 0.033$ , Kruskal–Wallis test); FgDP levels were significantly higher in the Trauma 30 group than the Control group ( $P < 0.05$ , Mann–Whitney  $U$ -test with Bonferroni correction). FgDP levels are presented in comparison with the density of the positive control, which was set at 100%.

**FIGURE 4.** Correlations between antithrombin and the ETP/FII and peak height/FII ratios. (A) Antithrombin was significantly negatively correlated with

peak height/FII ratio ( $\rho = -0.733$ ,  $P < 0.001$ ). (B) Antithrombin was significantly negatively correlated with ETP/FII ratio ( $\rho = -0.839$ ,  $P < 0.001$ ).

**FIGURE 5.** Representative thrombin generation curves. (A) Stimulated thrombin generation curve. Although the ETP was lower in the Trauma 0 group than the Control group, lag time and time to peak were shorter in the Trauma 0 group than the Control group, suggesting coagulation activation. Blue line: Control group; red line: Trauma 0 group. (B) Non-stimulated thrombin generation curve. Spontaneous thrombin bursts were observed in the Trauma 0 group but not the Control group, demonstrating the presence of circulating procoagulants in the systemic circulation. Blue line: Control group; red line: Trauma 0 group.

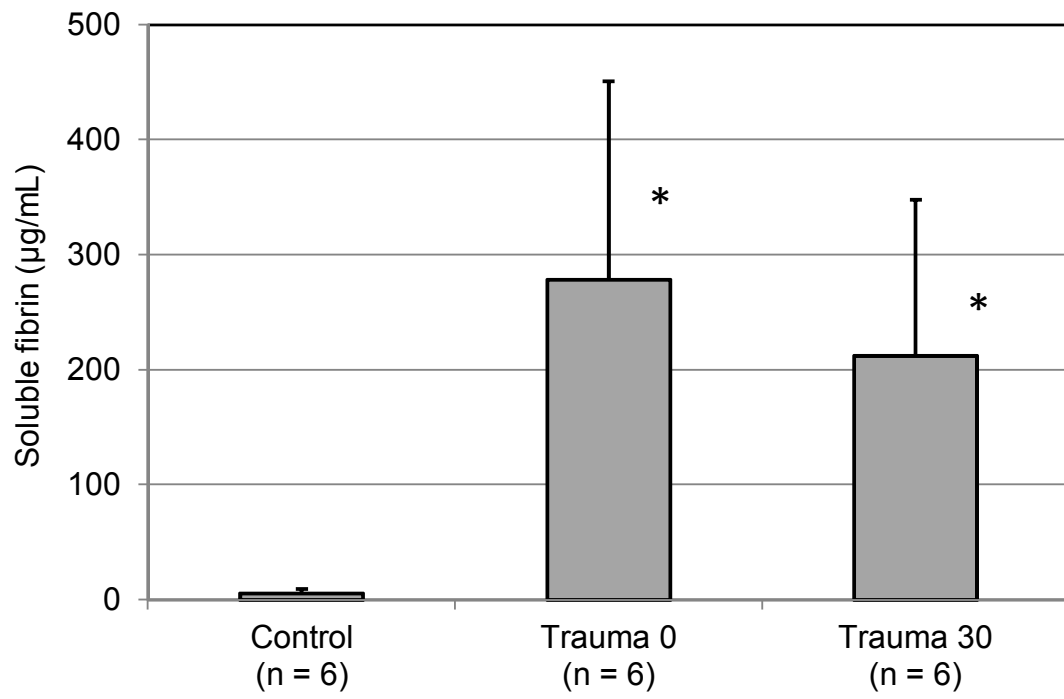


Figure 1

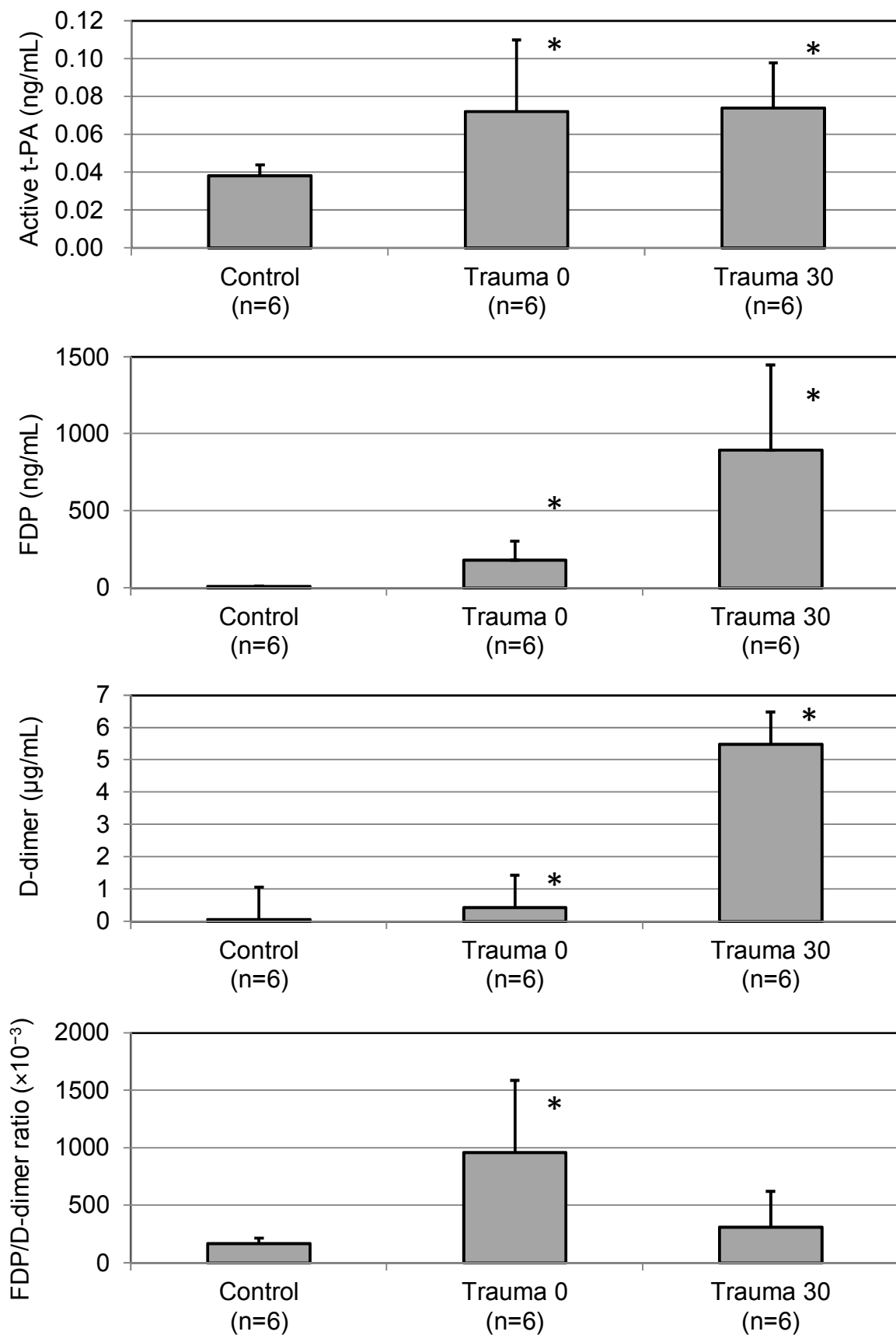


Figure 2

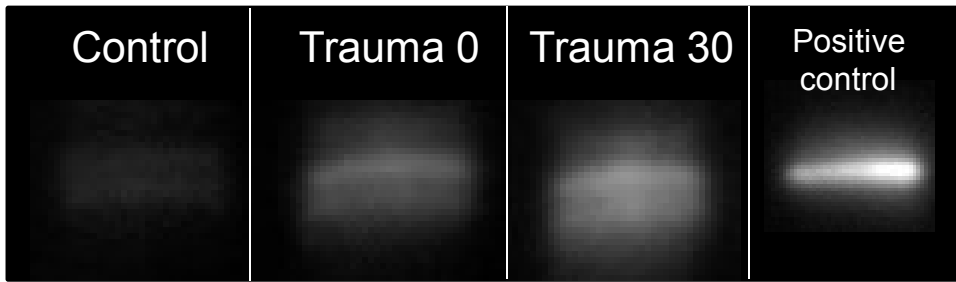


Figure 3

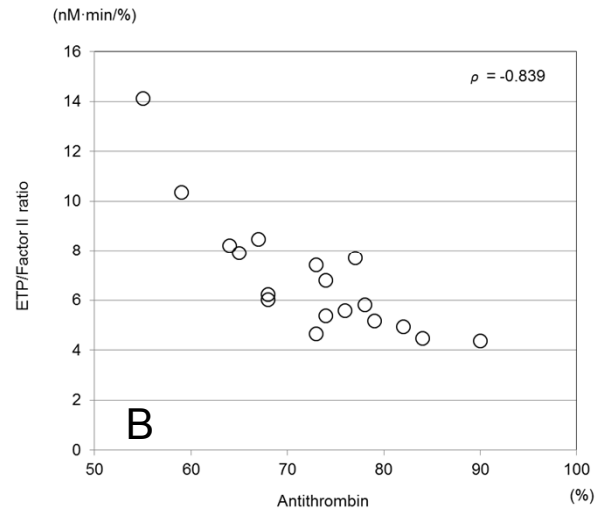
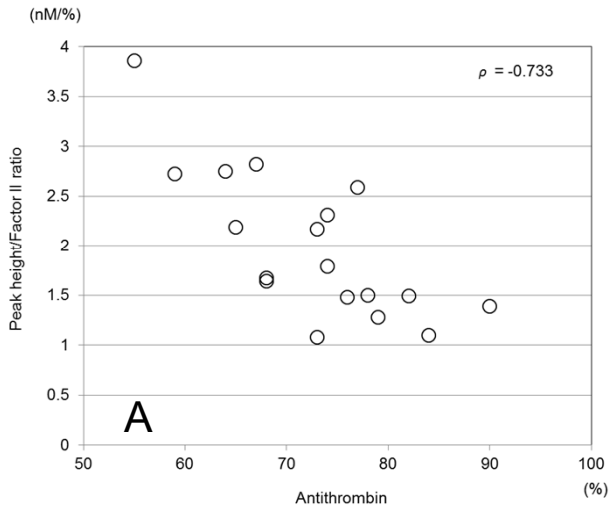


Figure 4

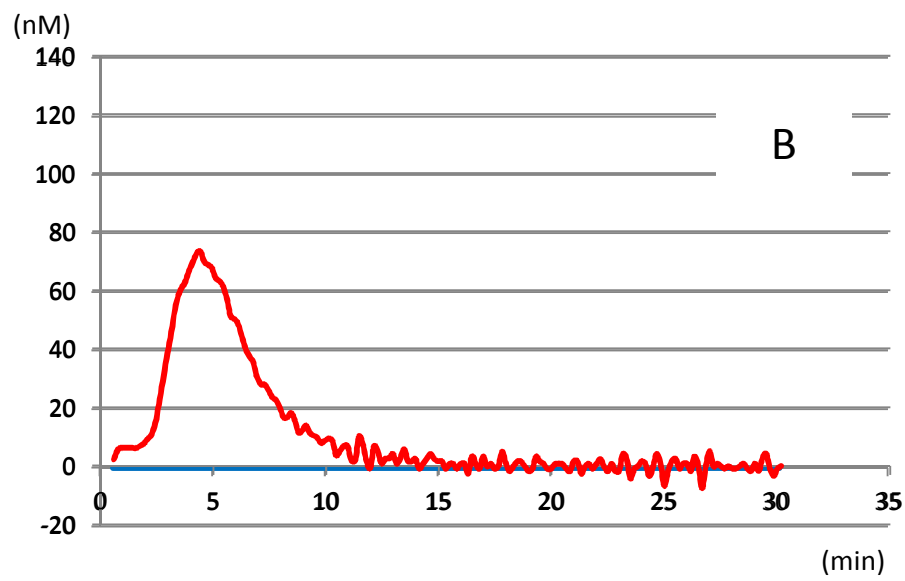
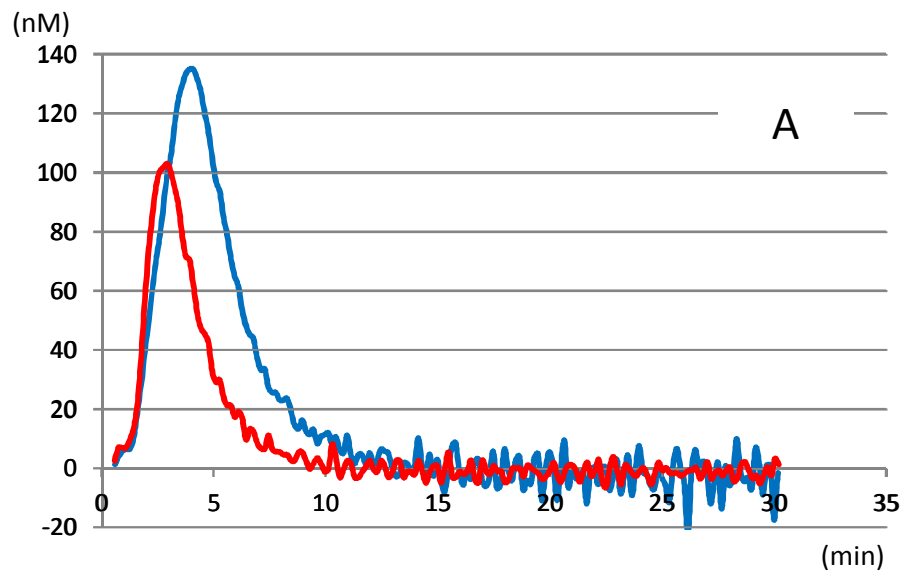


Figure 5

Table 1 General characteristics

	Control (n = 6)	Trauma 0 (n = 6)	Trauma 30 (n = 6)	P value
Body weight (g)	297 ± 15	309 ± 16	306 ± 21	NS
White blood cells (/μL)	4571 ± 1392	5913 ± 1208	5400 ± 1343	NS
Hemoglobin (g/dL)	14.9 ± 0.7	15.7 ± 0.7	15.3 ± 0.6	NS
Lactate (mmol/L)	0.94 ± 0.25	3.29 ± 1.18 *	2.53 ± 1.19 *	0.001
Creatine kinase (IU/L)	639 ± 226	2824 ± 1616 *	2870 ± 563 *	0.001
Lactate dehydrogenase (IU/L)	1077 ± 410	8062 ± 3812 *	12617 ± 6666 *	0.001
Mean arterial pressure (mmHg)				
0 min	134 ± 13	91 ± 19 *	97 ± 18 *	0.002
15 min	—	—	96 ± 17	—
30 min	—	—	103 ± 19	—

In the Control and Trauma 0 groups, arterial pressure was measured and arterial blood was drawn at 0 min. In the Trauma 30 group, arterial pressure was measured at 0, 15, and 30 min and arterial blood was drawn at 30 min.

NS, not significant. \*  $P < 0.05$  vs. Control, Mann–Whitney  $U$ -test with Bonferroni correction.