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*Original Article*

# Effect of defibrase on deep vein thrombosis following the surgical treatment of pelvic fracture

Minfei Wu[1], Shuquan Zhang[2], Yifu Sun[3], Rui Gu[3]

*[1]Department of Orthopedics, The Second Hospital of Jilin University, Changchun 130041, Jilin, China; [2]Department of Orthopedics, Tianjin Nankai Hospital, Tianjin 300100, China; [3]Department of Orthopedics, China-Japan Union Hospital of Jilin University, Changchun 130033, Jilin, China*

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**Abstract:** Objective: This study is to investigate the effect of defibrase on deep vein thrombosis (DVT) following the surgical treatment for pelvic fracture. Methods: This study included 40 patients who received surgical treatment for pelvic fracture. Blood samples were collected at 24 h before surgery, and 24 h and 72 h after surgery. Plasma levels of endothelin (ET), thromboxane $B_2$ ($TXB_2$), and 6-Keto-$PGF_{Ia}$ were measured with radioimmunoassay. Plasma D-dimer (D-Di) level was determined with using immune colloidal gold method. Results: For ET and $TXB_2$, in the control group, compared with 24 h before surgery, the plasma ET and $TXB_2$ levels were significantly elevated at 24 h after surgery, which were further increased at 72 h after surgery. In the defibrase group, the plasma ET and $TXB_2$ levels were significantly increased at 24 h after surgery, which were slightly declined 48 h later. Compared with the control groups, the plasma ET and $TXB_2$ levels were significantly declined in the defibrase group at corresponding time points after surgery. For 6-Keto-$PGF_{Ia}$ and D-Di, in the control group, the plasma 6-Keto-$PGF_{Ia}$ and D-Di levels were higher at 24 h after surgery than before surgery, which were further elevated 48 h later. In the defibrase group, the plasma 6-Keto-$PGF_{Ia}$ and D-Di levels were significantly declined at 24 h after surgery, which were further decreased at 72 h after surgery. Conclusion: Defibrase could significantly decline the incidence of DVT following the surgical treatment for pelvic fracture.

**Keywords:** Pelvic fracture, deep vein thrombosis (DVT), defibrase, endothelin (ET), thromboxane $B_2$ ($TXB_2$), 6-Keto-$PGF_{1a}$

## Introduction

Pelvic fracture accounts for 1-3% of the bone and joint injuries, the incidence of which is estimated at 20.2-35.2/100000 [1]. Patients always suffer a lot from pelvic fracture and subsequent surgical treatments, and the main complications of pelvic fracture include hemorrhagic shock, retroperitoneal bleeding, bladder/urethral injuries, and small intestine incarceration [2, 3]. These complications would significantly elevate the incidence of deep vein thrombosis (DVT) in patients, which might eventually lead to death [4, 5]. DVT refers to the blood clots forming in deep veins, which blocks the veins and induces chronic deep venous insufficiency. The incidence of DVT in orthopedics and neurosurgery is 15-40%, while the DVT incidence is 40-80% for severe trauma and pelvic fracture [6]. Traumatic fracture could cause vascular endothelial damages, which slow down venous blood flow and cause tissue edema, probably resulting in thrombosis. DVT could be found in the legs of 62-65% trauma-related death cases, and the risk of DVT is elevated along with the increasing trauma energy [7]. Therefore, thrombotic indicators might contribute to the early diagnosis and therapeutic treatments for the complications to reduce the injury mortality.

Endothelin (ET) is synthesized and secreted by endothelial cells, which has been well known for the strong vasoconstriction effect. Plasma ET level elevation might slow down blood flow and result in thrombosis [8]. Thromboxane $A_2$ ($TXA_2$) is generated during the arachidonic acid metabolism in platelets, which promotes platelet aggregation and induces blood vessel contraction [9]. Prostacyclin ($PGI_2$) is an antagonist

of thromboxane, and the declined synthesis of $PGI_2$ would also promote thrombosis [10]. Due to the extreme instabilities of $TXA_2$ and $PGI_2$, their hydrolysates, i.e., $TXB_2$ and 6-Keto-$PGF_{1a}$ are usually measured instead. Under normal circumstances, plasma ET, $TXA_2$, and $PGI_2$ levels stay in dynamic homeostasis, regulating coagulation and maintaining vascular tone and platelet function [11]. However, the dynamic equilibrium of ET, $TXB_2$, and $PGI_2$ would be destroyed by pelvic fracture and related surgical treatments. On the other hand, D-dimer (D-Di) is one of the specific products of the cross-linked fibrin degradation, which indicates the hypercoagulable state and secondary hyperfibrinolysis [4]. The secondary thrombosis following fibrinolysis would lead to posttraumatic D-Di elevation, and D-Di has been recognized as the exclusive diagnostic criteria for thrombotic diseases in recent years.

Defibrase is a venom preparation, the main pharmacological effects of which include reducing fibrinogen (FIB), inhibiting thrombosis, and improving microcirculation [12]. In this study, the effects of defibrase on DVT following the surgical treatment for pelvic fracture were investigated. The plasma levels of ET, $TXB_2$, 6-Keto-$PGF_{1a}$, and D-Di in the pelvic fracture patients were measured, before and after surgery. Clinical significance of defibrase in the prevention of DVT was also discussed.

## Materials and methods

### *Study subjects and grouping*

Totally 40 patients (27 males and 13 females; ages ranging from 35 to 40 years), who received the internal fixation surgery for pelvic fracture in our hospital, were included in this study. All these pelvic fracture cases were caused by road traffic accident trauma, with no other fractures. Pelvic fracture was confirmed with pelvic CT reconstruction, accompanied with surgical indications. These patients were associated with no history of coagulation disorders or systemic coagulation-related diseases (such as tumor and diabetes). Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of China-Japan Union Hospital of Jilin University.

These subjects were randomly divided into the control (n = 20) and defibrase (n = 20) groups. In the defibrase group, defibrase was administered by intravenous infusion every other day, for totally three times. The first-time dosage was set as 10 u, and 5 u was used for the other two times.

### *Determination of plasma levels of ET, $TXB_2$, 6-Keto-$PGF_{1a}$, and D-Di*

Totally 5 mL veinal blood samples were collected from these patients at 24 h before surgery, and 24 h and 72 h after surgery, respectively. After 30-min incubation at room temperature, the samples were centrifuged at 3000 r/min for 10 min. Plasma was separated, and then stored at -20°C until measurement.

Plasma levels of ET, $TXB_2$, and 6-Keto-$PGF_{1a}$ were measured with radioimmunoassay kits (Getein, Nanjing, Jiangsu, China), according to the manufacturer's instructions. On the other hand, plasma D-Di level was determined with using immune colloidal gold method (Getein).

### *Statistical analysis*

Data were expressed as mean ± SD. SPSS 13.0 software was used for statistical analysis, and *t*-test was performed for group comparison. $P < 0.05$ was considered statistically significant.

## Results

### *Plasma levels of ET in pelvic fracture patients before and after surgery*

Plasma levels of ET in pelvic fracture patients from the control and defibrase groups were determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Our results showed that, in the control group, compared with the measurement at 24 h before surgery, the plasma ET level was significantly elevated at 24 h after surgery ($P < 0.05$ v.s. control), which was further increased at 72 h after surgery ($P < 0.05$ v.s. 24 h after surgery) (**Figure 1**). On the other hand, in the defibrase group, compared with 24 h before surgery, the plasma ET level was significantly increased at 24 h after surgery ($P < 0.05$), which was slightly declined at 72 h after surgery (still higher than before surgery) (**Figure 1**).

Importantly, compared with the control groups, the plasma levels of ET were significantly declined in the defibrase group at corresponding time points (i.e., 24 h and 72 h) after surgery

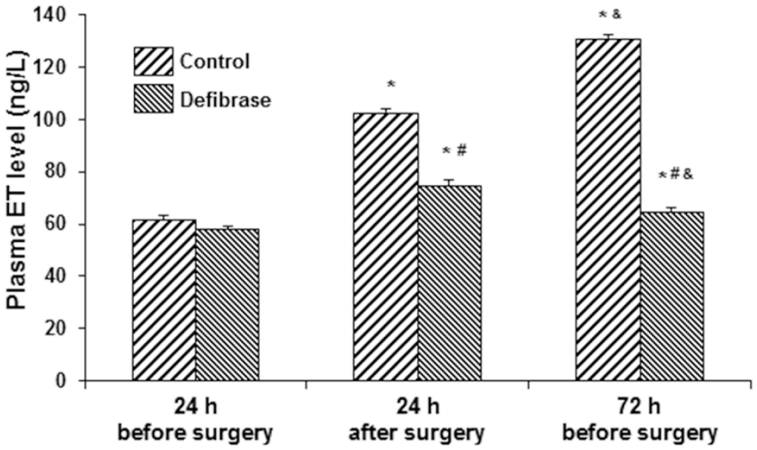


**Figure 1.** Plasma ET levels in pelvic fracture patients before and after surgery. Plasma ET levels in pelvic fracture patients from the control and defibrase groups were determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Compared with before surgery, $^{*}P < 0.05$; compared with control at corresponding time point, $^{\#}P < 0.05$; compared with 24 h after surgery, $^{\&}P < 0.05$.

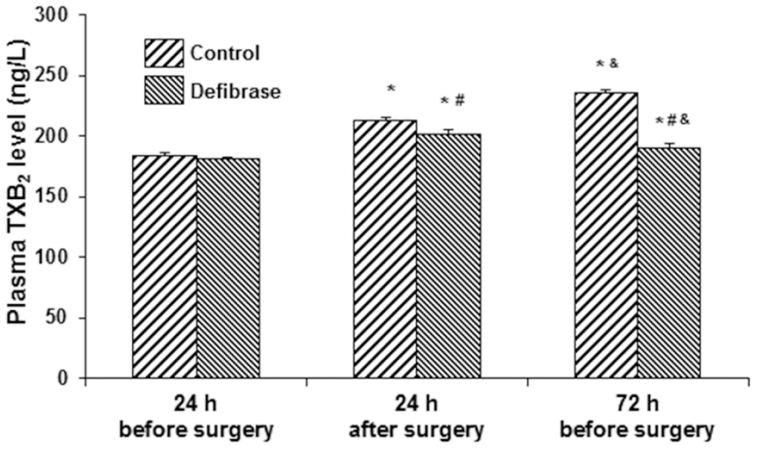


**Figure 2.** Plasma $TXB_2$ levels in pelvic fracture patients before and after surgery. Plasma $TXB_2$ levels in pelvic fracture patients from the control and defibrase groups were determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Compared with before surgery, $^{*}P < 0.05$; compared with control at corresponding time point, $^{\#}P < 0.05$; compared with 24 h after surgery, $^{\&}P < 0.05$.

(both $P < 0.05$). These results suggest that, compared with the control group, the defibrase treatment could significantly decline the plasma level of ET in pelvic fracture patients after surgery.

*Plasma levels of $TXB_2$ in pelvic fracture patients before and after surgery*

Plasma levels of $TXB_2$ in the pelvic fracture patients were determined with radioimmunoassay at 24 h before surgery, and 24 and 72 h after surgery, respectively. Similar results with plasma ET level were obtained for $TXB_2$. In the control group, compared with before surgery, the plasma $TXB_2$ levels were significantly elevated at 24 h and 72 h after surgery ($P < 0.05$), along with the increasing time. Moreover, in the defibrase group, compared with before surgery, the plasma $TXB_2$ level was significantly increased at 24 h after surgery ($P < 0.05$), which was then slightly decreased 48 h later ($P < 0.05$) (**Figure 2**). Compared with the control groups, the plasma levels of $TXB_2$ were significantly lower in the defibrase group at 24 h and 72 h after surgery, respectively (both $P < 0.05$). These results suggest that, compared with the control group, the defibrase treatment could significantly decrease the plasma $TXB_2$ level in pelvic fracture patients after surgery.

*Plasma levels of 6-Keto-$PGF_{1a}$ in pelvic fracture patients before and after surgery*

The 6-Keto-$PGF_{1a}$ is one of the anticoagulants synthesized and secreted by vascular endothelial cells, which exerts anti-platelet aggregation and vessel dilation effects. Determination of plasma 6-Keto-$PGF_{1a}$ levels in pelvic fracture patients before and after surgery with radioimmunoassay indicated that, in the control group, the plasma 6-Keto-$PGF_{1a}$ level was higher at 24 h after surgery than 24 h before surgery ($P < 0.05$), which was further elevated at 72 h after surgery ($P < 0.05$). On the other hand, in the defibrase group, compared with 24 h before surgery, the plasma 6-Keto-$PGF_{1a}$ level was significantly declined at 24 h after surgery ($P < 0.05$), which was further

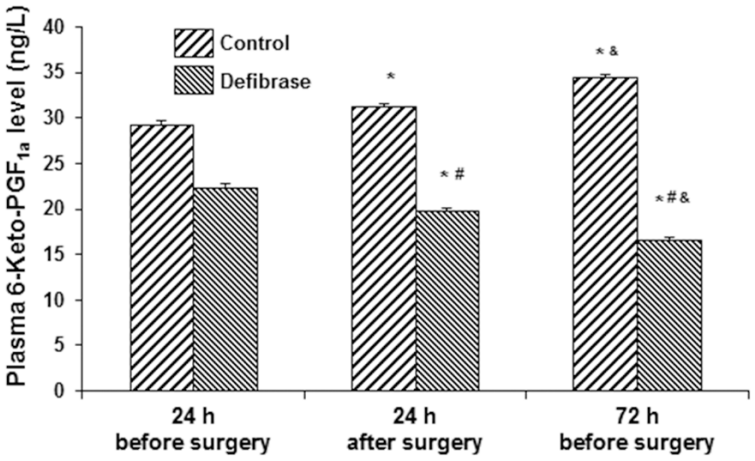


**Figure 3.** Plasma 6-Keto-$PGF_{1a}$ levels in pelvic fracture patients before and after surgery. Plasma 6-Keto-$PGF_{1a}$ levels in pelvic fracture patients from the control and defibrase groups were determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Compared with before surgery, $^{*}P < 0.05$; compared with control at corresponding time point, $^{\#}P < 0.05$; compared with 24 h after surgery, $^{\&}P < 0.05$.

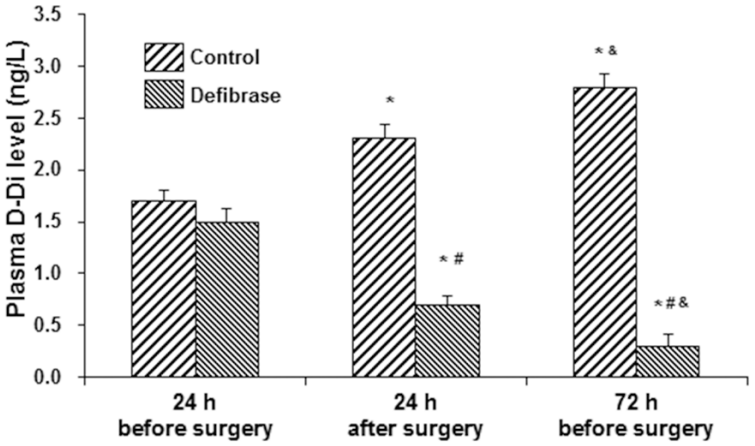


**Figure 4.** Plasma D-Di levels in pelvic fracture patients before and after surgery. Plasma D-Di levels in pelvic fracture patients from the control and defibrase groups were determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Compared with before surgery, $^{*}P < 0.05$; compared with control at corresponding time point, $^{\#}P < 0.05$; compared with 24 h after surgery, $^{\&}P < 0.05$.

decreased 48 h later ($P < 0.05$) (**Figure 3**). These results suggest that, defibrase could significantly decrease the plasma 6-Keto-$PGF_{1a}$ level in pelvic fracture patients after surgery.

*Plasma levels of D-Di in pelvic fracture patients before and after surgery*

Plasma D-Di levels in the pelvic fracture patients were also determined with radioimmunoassay at 24 h before surgery, and 24 h and 72 h after surgery, respectively. Our results showed that, in the control group, compared with before surgery, the plasma D-Di level was significantly elevated at 24 h after surgery ($P < 0.05$), which was further elevated at 72 h post-surgery ($P < 0.05$). However, in the defibrase group, compared with before surgery, the plasma D-Di level was significantly declined at 24 h after surgery ($P < 0.05$), which was then further decreased at 72 h post-surgery ($P < 0.05$) (**Figure 4**). These results suggest that, defibrase could significantly decrease the plasma D-Di level in pelvic fracture patients after surgery.

## Discussion

Acute DVT following surgery for pelvic fracture is relatively easy to identified and diagnosed by color Doppler ultrasound and venography, accompanied with clinical symptoms and signs. However, the early detection and prompt treatment for most DVT cases are still difficult due to the slow progression and occult early symptoms. Vascular endothelium plays an important and irreplaceable role in the regulation of vascular tone, vascular growth and development, monocyte adhesion, coagulation, and platelet aggregation [5]. ET is synthesized and secreted by endothelial cells, which has the strongest and the most lasting vessel contraction effect [13]. ET is released in case of vascular endothelial cell damage, and the elevated plasma ET level would slow down the blood flow and subsequently induce thrombosis [5, 9].

In addition to ET, vascular endothelial cell damage or destruction would also induce the

release of $TXA_2$, which exert significant vasoconstriction function [4, 14]. $PGI_2$ is an arachidonic acid metabolite, mainly generated by vascular endothelial cells. $PGI_2$ has been shown to be able to expand blood vessels, prevent platelet adhesion and aggregation, and prevent thrombosis [8, 15]. $PGI_2$ and $TXA_2$ perform substantially opposite functions in the body. Under normal circumstances, there is a dynamic homeostasis between $TXA_2$ and $PGI_2$, which contribute to the regulation of coagulation status and the maintenance of normal vascular tone and platelet function. A previous study has indicated a negative feedback regulation between ET and $PGI_2$ [16]. Moreover, the imbalance between $PGI_2$, $TXA_2$, and ET would result in vasoconstriction, slow blood flow, platelet aggregation, and diffuse microvascular thrombosis. These alterations might induce the pathogenesis and development of DVT [17].

In the present study, we collected and analyzed the blood samples from 40 patients who received surgical treatment for pelvic fracture. The plasma ET, $TXB_2$, 6-Keto-$PGF_{1a}$, and D-Di levels, and their roles in the predication of DVT after surgery, were investigated. Our results showed that, in the defibrase group, compared with before surgery, the plasma levels of ET, $TXB_2$, and D-Di were elevated, while plasma 6-Keto-$PGF_{1a}$ level was declined, after the surgical treatment. Opposite effects were observed for the control group. D-Di is a product from the cross-linked fibrin degradation, and it is a specific marker for the thrombin activation and secondary fibrinolysis. The level of D-Di has been shown to be elevated in acute VTE patients [18]. However, due to the high false positive rate, the sensitivity of D-Di in diagnosing DVT is 82-94%, and the specificity is 44-72%. Based on the above findings, we hypothesize that the elevated plasma levels of ET and $TXB_2$, together with the $PGI_2/TXA_2$ imbalance, induce the pathogenesis and development of DVT. Therefore, the early detection of ET, $TXB_2$, and 6-Keto-$PGF_{1a}$ is of great importance for the prediction of DVT and prevention of serious complications following surgical treatment for pelvic fracture.

In summary, our results showed that, in the defibrase group, compared with 24 h before surgery, the plasma ET and $TXB_2$ levels were significantly increased at 24 h after surgery, which were slightly declined 48 h later. Moreover, compared with the control group, the plasma ET and TXB2 levels were significantly declined in the defibrase group at corresponding time points after surgery. For 6-Keto-$PGF_{1a}$ and D-Di, in the defibrase group, the plasma 6-Keto-$PGF_{1a}$ and D-Di levels were significantly declined at 24 h after surgery, which were further decreased at 72 h after surgery. These results suggest that defibrase could significantly decline the DVT incidence. Our findings provide evidence for the application of defibrase in the treatment and prevention of DVT following the surgical treatment of pelvic fracture.

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## Disclosure of conflict of interest

None.

**Address correspondence to:** Rui Gu, Department of Orthopedics, China-Japan Union Hospital of Jilin University, No. 126, Xiantai Street, Changchun 130033, Jilin, China. Tel: 86-0431 8987 6939; E-mail: grjn001@163.com

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